December 1997

Marine Oils from Australian Fish:

Characterization and Value Added Products

Peter Nichols
Nick Elliott
Michael Bakes
Ben Mooney

FRDC PROJECT 94/115 Final Report

Nichols, Peter, 1956-
Marine oils from Australian fish:
Characterization and value added products

ISBN 0643 061533

1. Marine fish - lipids
I. Nichols, Peter, 1956-
II. CSIRO Division of Marine Research
III. Fisheries Research and Development Corporation (Australia)
IV. Title

TABLE OF CONTENTS

1. SUMMARY

Fish contain an array of oils, which vary markedly between species. The waste, by-catch and by-products from the Australian fishing industry can therefore be value-added to yield a range of marine oils. It is estimated that 100 000 tonnes per annum of the Australian fish catch goes to waste. Research from this Project (and its forerunner 91/77) has focused on oil from deep water and pelagic fish, and as a result of this work several companies have commenced production of marine oils for export and local use. Our research involved characterization of marine oils from Australian species, searching for new sources of commercially sought-after oils, development of new or refinement of existing processes suitable for Australian oils, and transfer of know-how to industry. Strong links exist with industry, giving an increased return for both the fishermen and oil processors, without increasing catch effort. Oils examined include: (i) wax esters derived from orange roughy, oreo dories and other fishes, (ii) shark liver oils containing squalene and diacylglycerol ethers, and (iii) triacylglycerol oils rich in essential omega-3 fatty acids. The oils are used as lubricants, in degreaser and hand cleaner products, in cosmetics and nutraceuticals.

The strengths of the Australian Marine Oils industry include: (i) relative sustainability of raw material (some countries show resources in decline), (ii) a clean and green image of the local resource, (iii) closeness to Asian markets, (iv) uniqueness of composition of several marine oils, (v) an increasing knowledge of marine oils and (vi) development of appropriate technologies. Opportunities exist based on these strengths and the Marine Oils industry has taken several products into the international market place. Together these features provide the Australian fishing and associated industries with the capacity to better utilize existing resources.

Wax ester oils
Research provided biochemical data on orange roughy oil and the waste obtained from processing, thereby assisting industry to better utilize the orange roughy resource. From data on the oil composition of orange roughy and its properties, a range of wax ester-derived degreaser, hand cleaner and other industrial products have been manufactured in Australia by BEKU Environmental Products Ltd. The oil from deep water oreos also contains high levels of wax esters and, it is feasible to use this oil in addition to that from orange roughy. We examined the composition of oil from other fish containing wax esters together with the composition and physical properties of wax ester oils produced by industry, including further value-adding oils to a high purity wax ester, the latter working with BEKU on process refinement.

Shark liver oils
Deep sea sharks are a significant by-catch of the orange roughy and other fisheries. Shark livers are large (20% of the total shark weight) and contain considerable quantities of oil which is often enriched in squalene. Squalene is used as a health-food or is hydrogenated to squalane, which is used in the pharmaceutical and cosmetic industries as a lubricant and cosmetic base. We have evaluated the squalene content of deep-sea shark species landed in Australian and other waters, and assisted industry in screening the squalene content of oils.

Research was completed on processing of crude oils so that the opportunity existed to export refined squalene, rather than crude shark liver oil. Several processes for separating and purifying squalene have been established and at present BEKU is the sole Australian manufacturer of squalene. In association with CSIRO, BEKU has further developed a diacylglycerol ether (DAGE) fraction from shark liver oil. This new product is also used in nutraceuticals. Technology has been developed for the preparation and purification of squalane (derived from squalene). Options for use

of by-products from the squalane purification process will need to be further examined.

Omega-3 polyunsaturated fatty acid (PUFA) oils

There is increasing scientific evidence that inclusion of fish-derived long-chain PUFA in the human diet reduces the incidence of coronary heart disease, stroke and other disorders (e.g. dyslexia, atherosclerosis, childhood asthma). Omega-3 PUFA oils are also thought to be beneficial for brain and retina development and function. Capsules of fish oils with high levels of the essential PUFA, eicosapentaenoic acid [EPA, 20:5(n-3)] and docosahexaenoic acid [DHA, 22:6(n-3)], are marketed overseas. Imported products (e.g. MaxEPA), with around 30% EPA plus DHA, have captured a small market in Australia. More recently omega-3 [also termed (n-3)] oils have been incorporated in other food items. For example, in Scandinavia oils are added to bread. Further processing can produce omega-3 oils containing higher levels of EPA and DHA (approx. 70-80%).

The level of EPA and DHA in oils from selected Australian fish, fish oils and capsules was determined. The demand for fish-derived or related (n-3) oils in Australian aquaculture is increasing. By determining the oil composition of Australian species (e.g. tuna, jack mackerel, southern sharks, snapper) and examining ways to add value to the crude (n-3) oils, industry will be better placed to identify alternative feedstocks and to take advantage of market opportunities. Other species examined include European carp, Patagonian toothfish, several temperate sharks and ling.

The growing interest in these oils has encouraged the establishment of technologies in Australia for adding value to the omega-3 oils. To date a process has been developed in the laboratory for purification of triacylglycerol-containing oils and scale-up of the process is underway in association with Nu-Mega Lipids, a joint venture between Clover Corporation and Heinz. The Nu-Mega Lipids initiative will utilize a resource that would otherwise be disposed of as waste and instead will produce refined tuna oil products. Research on enrichment technologies was performed with several products prepared in laboratory trials, all containing in excess of 90% omega-3 PUFA.

In addition to marine products research, a complimentary FRDC study (Project 95/122) was established to examine a wider range of Australia's commercial and non-commercial fishes for their nutritional composition, with an emphasis on oils. This research is due for completion in early 1998.

Antarctic Biota and microorganisms

Collaborative studies on lipids of microorganisms, zooplankton and fish from the Southern Ocean have increased the understanding of the biochemical and microbial processes of the Antarctic ecosystem. Specific lipids are used in the taxonomy of new Antarctic bacteria, including those able to synthesize potentially useful natural products (e.g. omega-3 and omega-6 PUFA). Lipids have been used to elucidate krill, zooplankton and other food-chain interactions, including those of commercial interest. Methodologies also have been transferred to aquaculture and environmental studies. For example, we have demonstrated the enrichment of omega-3 PUFA into rotifers using Antarctic bacteria. Techniques and processes developed by the marine oils industry can be transferred to biotechnological applications of Southern Ocean species.

The Australian Marine Oils industry has made rapid progress over the past 5 years and opportunities are now available to consolidate developments and commence new initiatives. Ongoing oil characterization and process oriented research will complement and strengthen existing industry initiatives and allow the Marine Oils industry to maximize returns on the present fish catch.

2. ABBREVIATIONS

AA	Arachidonic acid
AAOCS	Australasian Section of the American Oil Chemists' Society
AOCS	American Oil Chemists' Society
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DAGE	Diacylglycerol ether
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
EPA	Eicosapentaenoic acid
FFA	Free fatty acid
FRDC	Fisheries Research and Development Corporation
GC	Gas chromatography
GC-MS	Gas chromatography-mass spectrometry
GLA	γ-linolenic acid
HC	Hydrocarbon
LA	Linoleic acid
PUFA	Polyunsaturated fatty acids
SA	Stearidonic acid
SCO	Single cell oil
SFC	Supercritical fluid chromatography
SFE	Supercritical fluid extraction
ST	Sterol
TAG	Triacylglycerol
TGA	Therapeutic Goods Act
TLC-FID	Thin layer chromatography-flame ionization detection
UN	Unidentified
WE	Wax ester

3. BACKGROUND

Research undertaken by the CSIRO Division of Marine Research (formerly Divisions of Oceanography and Fisheries) Marine Products project has previously drawn together the combined expertise of CSIRO and industry. The objectives were to increase the value of the by-catch and by-products of Australia's south-eastern fisheries, in particular the larger, and economically important catches of orange roughy, oreos, jack mackerel, blue grenadier 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, oreo dories 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 is a need to characterize the oils derived from Australian species.

With the aid of previous FRDC funding (grant 91/77, Orange roughy and other marine oils: characterization and commercial applications), the Marine Products project 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 assisted a number of companies that established facilities for the production of oil and high grade meal from the south-east Australian fisheries.

Initial characterization of oils derived from commercially-landed Australian species and related samples was undertaken as part of the previous FRDC funded study (91/77). This work directly assisted Australian industry and resulted in the manufacture of value-added degreaser and other cleaning products from orange roughy oil.

A process to separate squalene from shark liver oils was also developed in collaboration with CSIRO Division of Molecular Science (formerly Chemicals and Polymers) during the initial FRDC funded study. The process was licensed for use by industry (Squalus, ITL Australia) for the production of squalene for the local and export markets. The purified export-grade squalene is currently worth approximately $25 per kilogram. It was proposed that Australia could initially export 20–50 tons annually (worth $0.5–1.25M). At whatever level is achievable, the opportunity exists for the Australian fishing and associated industries to gain a significant portion of the international squalene market.

Although omega-3 PUFA are abundant in most fish oils, to date no value-added omega-3 containing products have been manufactured in Australia. Rather, such products are still imported and crude fish oil is at times exported for value-adding. 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. Using omega-3 PUFA rich oils derived from Australian

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

4. NEED

Value-added marine oil products can be manufactured from both the waste generated during sea-food processing and the by-catch 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 by-catch 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.

Enhancing post harvest production of Australia's fisheries and converting what is now waste material into value-added by-products is required. Such products include squalene from shark liver oil, omega-3 PUFA from conventional fish oils, and specialty products from wax ester rich oils. The economic yields of the 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 studied to date. The research effort is needed to enable the small businesses involved in this industry to continue to be viable. For example, at the time of commencement of this project, discussions had been held with several companies on the potential to manufacture a value-added omega-3 PUFA-containing product in Australia. Industry cannot afford to undertake the research on its own, nor does it have the technology or expertise to undertake all the required research.

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 by-catch species and also on species (e.g. various tuna and mackerel) which offer potential for either increased catch or better utilization, including oil production and value-adding.

Further research and development is required on optimizing methods for refining squalene-containing, omega-3 PUFA containing and other marine oils, thereby increasing product value. One aim of the project was therefore to develop methods for obtaining and fractionating marine oils, including for example the use of supercritical fluid extraction (SFE) and other procedures. SFE techniques have the potential to overcome problems associated with more conventional techniques. For example, the use of toxic solvents is avoided (important for production of materials for the health, pharmaceutical and related industries), as is the use of elevated temperature. In combination, these features will minimize oxidation of sensitive PUFA and other components during processing.

5. OBJECTIVES

Research was designed to meet the needs of Australian industry and the primary objective was:

- To assist Australian industry develop new marine oil based, value-added products from existing or new fisheries including the by-catch and waste generated by the fishing and related industries.

To achieve this primary objective, specific tasks to be performed were:

- 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:
  1. identifying optimum sources of omega-3 polyunsaturated fatty acids and other specialty oils (e.g. diacylglycerol ethers from deep-sea and other sharks), and
  2. 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 will be broad-based and transferable between fish species and the developing field of single cell oils (SCO) derived from microorganisms. This aspect of the project will include evaluation and possible use of supercritical fluid extraction (SFE) and fractionation techniques.

5.1 After further consultation with FRDC prior to commencing the project, the following **Likely Achievements (see also below for Achievements for Australian industry)** were proposed:

- further assessment of industry needs, through personal contact or letters
- completion of survey of oreo dory oil composition
- survey of the oil composition of southern sharks, including possible changes with season and other factors
- provision of assistance to industry for the calibration of hand held refractometers and other devices which are used to assess shark liver oil quality
- examination of the oil composition of shark liver oil for species from NT and WA
- development of laboratory methods for enrichment of diacylglycerol ethers from shark liver oil
- study of the omega-3 PUFA composition of Australian species; where appropriate changes with environmental and other factors will be examined
- pilot examination of existing and new technologies (including SFE) for enriching omega-3 PUFA from fish oil
- pilot examination of existing and new technologies (including SFE) for extracting and enriching shark liver oils.

5.2 **Likely achievements of project for Australian industry:**

- industry will be provided directly with advice, assistance and analyses of oil and related samples
- expansion of the data base on the composition of marine oils from Australian fish
- development or provision of assistance to industry for the development of new technologies for adding further value to new oils
- transfer of such technologies, related advice and other information to industry. For example, negotiations will continue to seek commercial partners for methods under development that purify omega-3 oils.

During year 1 of the project, research was performed as stated above in the objectives. In submission of the application for ongoing funding after completion of year 1, it was noted that the objectives remained as in the original proposal.

For consideration of the ongoing application of project 94/115, the FRDC Board requested a rigorous risk assessment and consideration of the commercially competitive international environment in which the local producers must operate.

The further input as requested by the Board included:
1. technical assessment regards achievements of objectives
2. analysis of environmental factors impacting on the marine oils industry
3. assessment of issues on a more specific/micro level.

A copy of the Risk Assessment Report as prepared at the completion of year 1 of Project 94/115 in mid 1995 is attached (Appendix B).

6. METHODS

Fish, oil and related materials (including SCO from microorganisms) were obtained from fishers, processors, manufacturers, CSIRO Division of Marine Research (formerly Division of Fisheries, overseen by Dr. N. Elliott) and other sources.

Lipid (oil) and fatty acid analyses were carried out by FRDC-funded scientists Mr. M. Bakes and Mr. B. Mooney under the supervision of Dr. P. Nichols in the organic chemistry laboratories of the CSIRO Division of Marine Research (formerly Division of Oceanography). Additional laboratory assistance was provided by other CSIRO staff working on related scientific projects.

Oil was extracted from samples using the Bligh and Dyer chloroform-methanol-water extraction scheme. The extracts were partitioned with purified water (Milli-Q) to remove salts and water soluble material. Lipids were recovered in the lower chloroform phase, the solvents removed under vacuum, and stored under nitrogen at –20°C until analysis.

Supercritical fluid extraction techniques were designed from the scientific literature and experiments undertaken during the course of the project. A number of recent review articles exist for application of SFE and SCF to omega-3 PUFA materials. However, developmental work was required for adaptation of the methods to oils derived from Australian fish and for application of these techniques to shark liver oil. Limited work had been performed on the isolation of squalene from olive oil, and it was believed aspects of the technology would be transferable to shark liver oils.

Portions of the total lipid extracts were analysed with an Iatroscan Mark V TH-10 TLC-FID analyser to determine the concentration of major lipid classes. Total fatty acids, alcohols, glyceryl ether diols and sterols were obtained by alkaline saponification or, in the case of fatty acids, directly as methyl esters by acid-catalyzed transesterification. Samples were analysed using capillary gas chromatographs (GC) equipped with flame ionization detectors and automatic injectors. A nonpolar methyl silicone (HP1) fused-silica capillary column was used to identify and quantify the many fatty acid isomers and other components present. Selected samples were also analysed on a polar (e.g. BP20) capillary column. Data were acquired, processed and manipulated using MS-DOS based personal computers and appropriate chromatography (DAPA) and spreadsheet software packages (Excel). Lipid samples were also analysed by gas chromatography-mass spectrometry (GC-MS) to confirm component identifications.

Further details on experimental methods employed, including sample collection and preparation, are provided in the appended scientific papers (Appendix D).

Processes for fractionation and therefore value-adding of marine oils were examined, developed, or modified and refined during the project. Details are documented in various technical reports prepared during the project (Appendix A).

7. RESULTS

During the previous FRDC funded project (91/77), strong ties were established with several companies involved in fish processing or value-added fish by-products. These included for example, BEKU Environmental Products Ltd who we have assisted with the development of and provision of high quality analytical data for marine oil-based degreasers, cleaning and other products based on wax ester oils; Clover Corporation, Nu-Mega Lipids, HRL Technology and others who we have provided new compositional data on species containing omega-3 oils, and in addition, processing technology; BEKU, Scales, Squalus and HRL Technology and others on the development of shark liver oil, squalene, squalane, diacylglycerol ether and other products. As the fishing and associated industries have become more aware of our research, we have also responded to many requests for information from fishers and processors throughout Australia. Progress on project objectives and transfer of know-how and technology to Australian industry follows in this section.

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

- Preparation of reports for industry on (i) specific oil analyses and/or (ii) development of processing technologies.
- Direct personal communication to industry.
- Scientific presentations at conferences, workshops and seminars.
- Manuscripts in national and international journals.
- Where appropriate, for processes developed during the course of the project and related studies, protection of results (e.g. via patents or suitable mechanisms) has occurred (see also intellectual property section).

Transfer of results was also facilitated by several media releases and associated national publicity received during the project. Appendix A provides a list of publications and reports on marine oils over the past 3 years.

7.1 Assessment of industry needs, through personal contact or letters.

Close ties continued with a number of companies as outlined in the original proposal and as stated above. The project continues to interact strongly with industry and on a regular basis new clients approached the project team with requests for information, advice and/or detailed analysis of oils being produced or under consideration of production in Australia (see section 7.9, ***Achievements of Project for Industry***). Industry was supportive of the marine oil research performed during the project and representative letters of support are attached (Appendix E).

7.2 Survey of oreo dory, orange roughy and myctophid oil composition.

Wax ester containing degreaser and hand cleaner products have been produced since the initial discovery of the chemical composition and physical properties of orange roughy oil as part of project 91/77. The products were initially based exclusively on orange roughy oil. During the

course of project 94/115, further wax ester based products (e.g. cutting fluid, Appendix F) have been developed by BEKU Environmental Products working in association with CSIRO. We also have examined other species for similar marine-derived wax ester containing oils.

Analyses were completed on the oil, fatty acid and fatty alcohol composition of the major oreo species taken from the south-eastern trawl fishery. Further to publication of an article in Australian Fisheries in 1994, a more comprehensive paper was published in 1995 in the international journal Comparative Biochemistry and Physiology (Appendix D). The summary of this study follows.

Variation in lipid composition of some deep-sea fish (Teleostei: Oreosomatidae and Trachichthyidae)

The lipid, fatty acid and fatty alcohol compositions were determined for muscle samples from six 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. Similar analyses were carried out on the muscle and swim bladder of the orange roughy *Hoplostethus atlanticus* from both Australian and North Atlantic waters. Orange roughy is currently a major commercial species in southern Australia and is a new fishery in the North Atlantic; there are four species of oreo of increasing commercial significance in Australia due to orange roughy quota reductions. It is therefore necessary to determine if the oreo fishing industry is capable of supplementing the current orange roughy requirements with respect to muscle and oil demand. In the oreos, the mean lipid content ranged from 0.5 to 3% of wet weight, with a mixed lipid composition including wax ester, triacylglycerol, sterol and polar lipid. The ratio of the monounsaturated fatty alcohols 22:1 to 20:1 allowed samples from the two geographical regions to be distinguished. Total wax ester in muscle from North Atlantic male orange roughy was much higher than in Australian fish (27 vs. 8.5% wet weight, respectively); females from both locations contained similar amounts of wax ester (4.5 vs. 3.3%, respectively). Selected swim bladders from North Atlantic and Australian orange roughy show similar wax ester content (90 vs. 82%, respectively). 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). Indeed differences exist between oreos from the two locations, and this requires further investigation. With respect to the nutritional value, the oreos are more attractive than the orange roughy, however, PUFA levels are lower compared with other popular species.

Another fish species with high levels of wax ester is the myctophid *Electrona antarctica.* The lipid composition of this southern ocean species was examined in a collaborative project with visiting scientist Prof C. F. Phleger of San Diego State University, California. A scientific paper was published in 1997 on the findings (Appendix D). A summary of the research follows.

High levels of wax ester in the Myctophid fish *Electrona antarctica*

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

The project also provided advice and analytical support to BEKU Environmental Products Ltd during pilot scale trials to produce an Australian-manufactured high purity wax ester product. A wax ester product of >99% purity was achieved. The opportunity now exists to produce a highly purified wax ester oil for use by cosmetic manufacturers.

7.3 The oil composition of southern sharks, including possible changes with season and other factors.

Analyses were performed on the liver oil content and composition of a range of southern and deep-sea sharks, and reports were prepared for industry (see Appendix A; copies of reports are held at the CSIRO Marine Laboratories, Hobart). Results were presented in late 1994 at the PACON meeting in Townsville and at the international Pacifichem conference in Honolulu. Newspaper and other popular articles and media coverage were obtained for project 94/115 and FRDC after the Townsville PACON presentation and following a press release in 1995 and again in 1996 and 1997 associated with media coverage for Project 95/122. Results were presented in poster form in July 1996 at the Australian Marine Sciences Association conference held in Hobart. A copy of the poster (A4 size) is appended (Appendix C). This poster was also presented in July 1996 at the 2nd World Fisheries Congress in Brisbane, at the Inaugural workshop of the Australasian Section of the American Oil Chemists Society (AAOCS, Canberra, February 1997) and at the AOCS National Meeting in Seattle in May 1997. Two scientific papers were published in 1995 in the conference proceedings from the PACON meeting and in Comparative Biochemistry and Physiology (Appendix B). The summaries of these two papers follow.

Value-added products from Australian shark liver oils

The by-catch and the waste generated by the Australian fishing industry are a resource with considerable economic potential. Scientific research agencies have worked collaboratively with industry to establish new processing techniques to isolate valuable lipids, including hydrocarbons (squalene), from the livers of deep-sea sharks. An increasing global demand for some of these specialized purified chemicals (e.g. machine lubricants, health products, skin moisturizers and other products) and rising costs of waste disposal have encouraged research in this area. The value of a previously wasted resource has now been identified as a commodity worth more than one million dollars (per annum) to Australian suppliers, based on the market demand, price and the current catch rates.

Lipid, fatty acid and squalene composition of liver oil from six species of deep-sea sharks collected in southern Australian waters

The liver oils from the deep-sea sharks *Somniosus pacificus* (Pacific sleeper shark), *Centroscymnus plunketi* (Plunket shark), *Centroscymnus crepidater* (Long-nose velvet shark), *Etmopterus granulosus* (Lantern shark), *Deania calcea* (Platypus (Brier) shark) and *Centrophorus scalpratus* (Endeavor shark) were analysed to describe their lipid, fatty acid and squalene compositions. The major lipids in all species of deep water shark were diacylglycerol ethers and triacylglycerols, and the major hydrocarbon was squalene (Figures 1 and 2). Only trace levels of polar lipids were detected. Monounsaturated fatty acids ($C_{16:1}$, $C_{18:1}$, $C_{22:1}$ 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 diol profiles. 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.

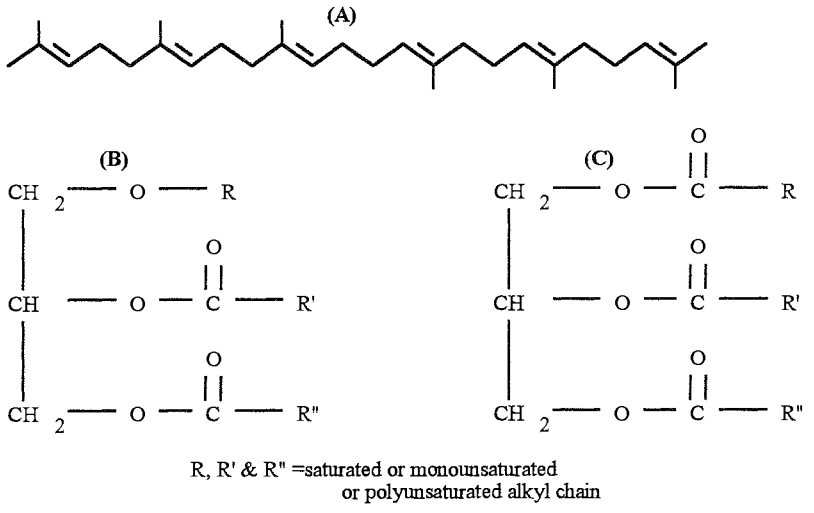


Figure 1. Structure of (A) squalene, (B) diacylglycerol ether (DAGE), and (C) triacylglycerol.

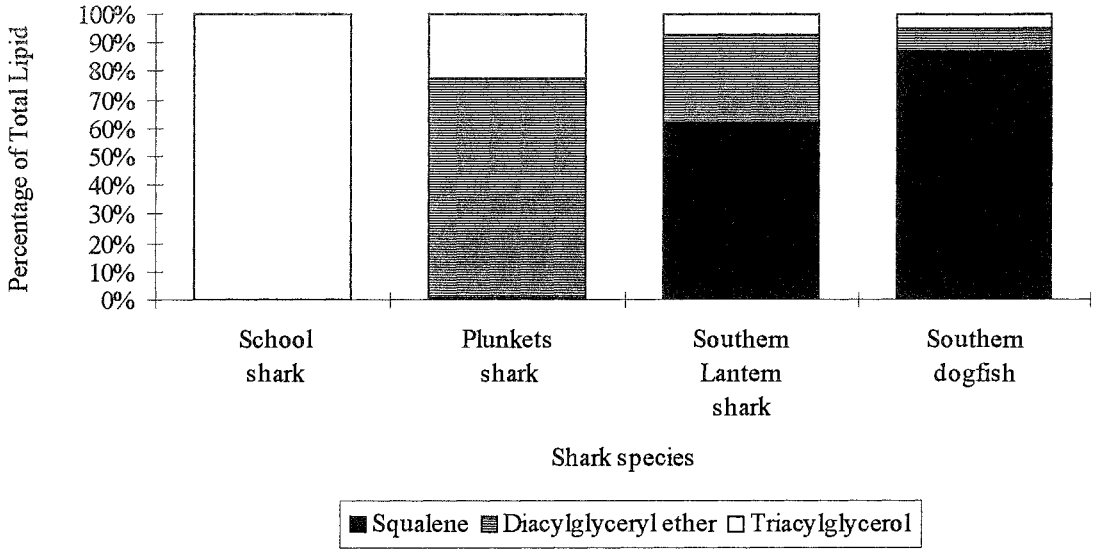


Figure 2. Lipid class composition (determined by TLC-FID) of liver oils from selected Australian deep-sea and other (school) shark species.

In collaboration with Brad Wetherbee of the University of Hawaii, analyses were completed on a survey of liver oil from a range of deep sea shark species examining the effect of water depth, size and other factors on oil composition. The sharks were collected from New Zealand waters. A summary of the results follows (see also Table 1).

Lipid Composition of Deep-sea Sharks: Variation with size and other factors

Deep-sea sharks approach neutral buoyancy through means of a large, oily liver that contains large amounts of low-density lipids, primarily squalene and diacylglycerol ether (DAGE) (Figure 1). As an animal increases in size and matures sexually, many biochemical changes take place within the animal. It was hypothesized that maintenance of neutral buoyancy in deep-sea sharks involves fine-scale changes in the chemical composition of liver oil as individuals grow and develop. To test this hypothesis the lipid composition of liver oil of individuals of different sizes and sex for around 200 specimens of species of deep-sea sharks collected from the Chatham Rise, New Zealand were compared. The composition of liver oil varied between, and among species (Table 1). Several species contained large amounts of squalene and DAGE, whereas only traces of these lipids were present in other species. There was an inverse relationship between the amount of squalene and DAGE in liver oil, and squalene content tended to decrease as sharks increased in size. Species with high squalene levels (>80%) in liver oil were not abundant on the Chatham Rise, as is observed in Tasmanian waters. However, DAGE levels were elevated in many species, and increasing commercial interest is occurring for oils rich in this lipid fraction. Maintenance of neutral buoyancy in deep-sea sharks is a dynamic process that involves changes in the composition of low density liver lipids as the sharks increase in size.

Table 1. Oil composition of shark liver oils. Data are the mean of 3 to 84 specimens, except for *Squalus acanthias* and *Hexanchus griseus* where only one specimen was available.

Species	Common name	Percentage composition				
		HC	WE	DAGE	TAG	FFA
Etmopterus granulosus	Southern lantern shark	54.7	0.6	35.0	9.0	0.8
Centroscymnus crepidater	Long-nose velvet shark	68.1	0.2	21.6	9.6	0.4
Centroscymnus plunketi	Plunket shark	0.7	0.1	91.9	6.6	0.7
Centroscymnus owstoni	Owstons dogfish	54.0	0.4	22.9	22.4	0.2
Deania calcea	Platypus shark	66.5	0.1	23.5	9.1	0.8
Dalatais licha	Black shark	80.2	0.5	18.4	0.5	0.4
Centrophorus squamosus	Leafscale gulper shark	55.2	0.1	28.5	15.9	0.3
Squalus acanthias	White-spotted spurdog	0.0	0.0	12.4	87.4	0.2
Hexanchus griseus	Sixgill shark	0.5	0.4	70.3	28.7	0.0
Aprisurus species A	Freckled catshark	0.1	0.2	0.2	99.3	0.3
species C	Fleshynose catshark	0.2	0.1	0.1	85.7	0.7
species E	Bulldog catshark	28.1	0.3	4.7	66.2	0.6

HC = hydrocarbon (predominately squalene), WE = wax ester, DAGE = diacylglycerol ether, TAG = triacylglycerol, FFA = free fatty acid

Analyses were conducted of liver oil from deep sea sharks to examine the inter-sample variation in the oil composition over 18 months (n=6). Oil was extracted and supplied by a commercial oil processor. Variations in oil composition were observed over the study period (squalene, 22% to 64%; DAGE, 23% to 52% ; TAG 11% to 25%). Once again, an inverse relationship between the amount of squalene and DAGE in the liver oils was observed. However, insufficient catch information was available from the processor to determine the effect of season versus other environmental (temperature, depth, location) or biological (species, sex, size) factors. At this stage it is believed to be unlikely that seasonal variation is a major factor in the inter-sample variation, and will have little influence for commercial operations targeting liver oils from deep sea sharks. Rather, the key factor is species composition.

Analyses were completed and a manuscript examining the lipid, fatty acid and vitamin composition of school and gummy shark liver oils was prepared for publication (Appendix D). Aspects of these results were presented at the Inaugural workshop of the Australasian Section of the American Oil Chemists' Society (Canberra, February 1997; Appendix D). Results were also presented in poster form at the same meeting. The summary of this study follows.

Docosahexaenoic acid-rich liver oils from temperate Australian shark livers

The livers from the two main commercially-targeted shark species in southern Australia (*Mustelus antarcticus*, gummy shark and *Galeorhinus galeus*, school shark), together with *Squalus acanthias* (white-spotted spurdog), were analysed for oil and fatty acid composition. The yield of oil from the liver was 30 to 64% (wet weight) for *M. antarcticus* and 50 to 53% for *G. galeus*. Lipid classes were determined by thin-layer chromatography with flame ionization detection (TLC-FID), with the major lipid in school (Figure 2) and gummy shark being triacylglycerol (≥95%). Minor lipids were polar lipid, wax ester, sterol (mainly cholesterol) and free fatty acid. This finding is in contrast to results for liver oils from deep sea sharks; the latter contain elevated levels of squalene and DAGE. Long-chain omega-3 polyunsaturated fatty acids (PUFA) accounted for between 33 and 39% of the total fatty acids in both species and docosahexaenoic acid (DHA) levels were between 13 and 18%. In comparison with imported commercially-produced oils currently marketed in Australia, the oil from the livers of *M. antarcticus* and *G. galeus* and other shark species may be an attractive source of omega-3 fatty acids, specifically DHA, for direct use and/or for further value adding, thus increasing the return to fishers.

7.4 Assist industry with the calibration of hand-held refractometers and other devices which are used to assess shark liver oil quality.

During year 1, a literature survey was conducted and two hand-held refractometers were trialled. Literature summaries and our results from the laboratory trials were made available to industry. Hand-held refractometers can provide a useful first-round measure of squalene content in liver oils. In passing results on to industry, it was noted that some oils known to be low in squalene content gave refractive index readings corresponding to higher squalene values than expected. The need to verify squalene results using laboratory-based techniques other than refractometers was highlighted and also communicated to industry. No further research with refractometers was conducted during the project.

7.5 Examination of the oil composition of shark liver oil for species from NT and WA.

Three samples of shark liver oil from the NT and WA were obtained through commercial sources. The WA sample of liver oil from *Centrophorous uyata* contained very high levels of squalene. In fact, liver oil from *Centrophorous uyata* contained the highest squalene level (85%) found to date for liver oils obtained from deep-sea shark species analysed from Australian waters. The two samples of liver oil from NT samples (sand bar and tiger shark) contained either very low levels or no squalene. Rather, the oils contained high levels of triacylglycerol. These TAG oils also contained PUFA such as DHA, and as noted above for liver oils from school and gummy sharks and other temperate shark species may be suitable for further value-adding.

7.6 Develop laboratory methods for enrichment of diacylglycerol ethers from shark liver oil.

A laboratory procedure (involving column chromatography) was developed to isolate diacylglycerol ethers from shark liver oil. The method was used to obtain a pure standard for use

by the project.

Further research on diacylglycerol ethers derived from shark liver oils was conducted in association with BEKU. A fraction containing diacylglycerol ethers and triacylglycerols is the main by-product from the production of squalene from shark liver oil by BEKU. This by-product is commonly termed the diacylglycerol ether (DAGE) fraction and is used as a health supplement. In May 1997, "Shark Liver Oil" (Solomon et al., Kensington Books, New York) was published; the book reported literature examples of the use of shark liver oil against a range of disorders and also noted aspects of Australian research. BEKU has exported the DAGE fraction, with future prospects also promising (Appendix F). The potential to maximize return on the production of squalene from shark liver oil will be greatly enhanced by the additional sale of the by-product DAGE fraction.

Closer examination of the composition of the BEKU and several other commercially available diacylglycerol fractions reveals differences between the oils that may be of significance in product development. Varying levels of the three main components were present in the DAGE oils analysed: DAGE, 34-74%; triacylglycerol, 17-32%; squalene, <0.5-35%. The significance of the levels of the three main components, and their relative proportion, for use in animal and/or human products remains to be determined. However, knowledge of the composition of similar fractions in use or under trial by industry will aid any Australian initiatives with this fraction. When such product information and knowledge of biological uses and effects is available, it may be possible to modify existing factory procedures to change or tailor product composition.

A glyceryl ether diol fraction can be produced from DAGE by cleavage of the fatty acid side-chains using base saponification or other procedures. The glyceryl ether alkyl chains are comprised of: 18:1 (main component), 18:0, 16:0, 16:1, 14:0, 14:1 and 20:1. The distribution of alkyl chains is generally less complex than for the fatty acids derived from the DAGE and triacylglycerol fractions (Appendix D: Bakes and Nichols 1995 and references therein).

The theoretical yield of glyceryl ether diol from pure DAGE is approximately 32% (calculated assuming an average alkyl chain length of C_{18}). Therefore, for a DAGE fraction containing 66% DAGE, the theoretical yield would be 21%. Losses would be expected during processing and a practical yield of 10-15% or less may be realistic. For fractions containing lower levels of DAGE, the theoretical and practical yields are lower again.

Laboratory trials were conducted to produce the glyceryl ether diol using a commercial DAGE fraction. The reaction was successful under the conditions employed, with all the starting DAGE and TAG present being converted to glyceryl ether diol and fatty acids (Figure 3).

Difficulties were encountered with the separation and low recovery of the glyceryl ether diol. These observations may be partly attributed to the presence of squalene in the starting DAGE fraction. From these results and the above considerations, the overall economics do not presently support the production of a shark liver oil-derived glyceryl ether diol product (based on access to existing human health markets). However, should sufficient market interest (and accompanying product value) exist for a glyceryl ether diol product, further research and development may overcome some of the difficulties encountered to date.

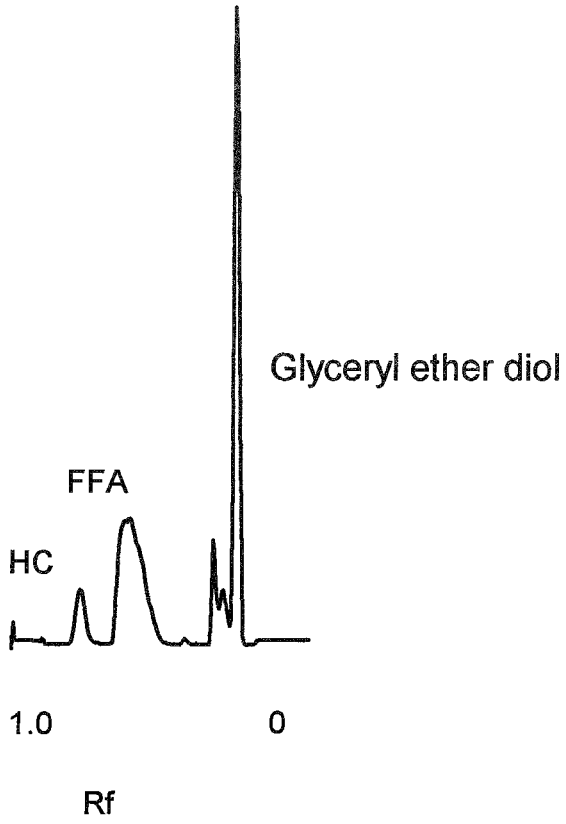


Figure 3. TLC-FID chromatogram of reaction products from treatment of a commercial DAGE fraction. HC denotes hydrocarbon (squalene). FFA denotes free fatty acid.

A report detailing analytical methods used for quantitative determination of DAGE and glyceryl ether diol, including alkyl side-chain distributions, was prepared. The report was made available to Australian industry and overseas parties involved in product development with Australian shark liver oils.

The oil composition of a range of zooplankton was examined in collaboration with Prof C. F. Phleger from San Diego State University. A possible new source of DAGE was discovered in one pteropod species. As the deep-sea shark resource may be showing signs of overfishing in selected locations, harvest of pteropods therefore might be an alternate source of DAGE. *Clione limacina* accounts for up to 80% of the total Gymnosome population in the North Atlantic and is so common it is a food of the Greenland Right whale and some fishes. The economics for harvesting *Clione* would appear unattractive. However, culture of this species, including as a direct source of DAGE or for use as a source of genetic material for possible transgenic application, may have some potential. The lipid profile results for *Clione* and other pteropods have been collated and have been presented at an international conference by Prof. Phleger. A scientific manuscript describing these results was published (Appendix D) and a summary of the research follows.

Southern Ocean Pteropods; A Potential Buoyancy Role for Diacylglycerol Ether

Clione limacina, a Southern Ocean Pteropod (order Gymnosomata) contains 28% diacylglycerol ether (DAGE) whereas the Pteropod *Limacina helicina* (order Thecosomata) lacks DAGE. The alkyl glyceryl ether diols of *Clione* DAGE are dominated by 16:0 (62%) and 15:0 (22%) which contrasts with deep-sea shark liver DAGE, dominated by 18:1 diol. The fatty acid profiles of *Clione* and *Limacina* are similar (28-32% polyunsaturated, 26-34% monounsaturated) as are the sterols which include 24-methylenecholesterol, transdehydrocholesterol, cholesterol and desmosterol. This finding probably reflects the fact that *Limacina* is the major food source for *Clione*. *Spongiobranchaea australis*, another Southern Ocean Pteropod (order Gymnosomata) has 0.9-1.7% DAGE, but has less lipid (3.3-4.8 mg/g lipid, wet weight) than *Clione* (50.8 mg/g lipid, wet weight). We propose a buoyancy role for DAGE in *Clione* since *Limacina* has bubbles for flotation which *Clione* lacks; DAGE provides 23% more uplift than triacylglycerol at a sea water concentration of 1.025 g/ml.

7.7 The omega-3 PUFA composition of Australian fishes.

PUFA have received much attention in recent years, particularly omega-3 (or n-3) PUFA, due to their increased role as a medicine in the protection against coronary and ischemic heart disease, in the treatment of malaria, arthritis, as treatment for the lowering of blood pressure and reducing hypertension and against other disorders. Studies on the dietary intake of these fatty acids is ongoing in many laboratories. Omega-3 PUFA have also received interest from aquaculture and related industries, particularly in the rearing of larval fishes.

These findings have generally enhanced the image of fish as a healthy food for consumers. Capsules of fish oils that contain high levels of the essential PUFA, EPA [20:5(n-3)] and DHA [22:6(n-3)], are marketed aggressively internationally, and have captured a small market in Australia (e.g. MaxEPA and related products). There is an increasing body of evidence that DHA plays an important role in infant development and is active against a range of disorders. Animal studies conducted by the CSIRO Division of Human Nutrition have demonstrated that contrary to popular assumption, DHA rather than EPA is likely to be a principal active omega-3 fatty acid for a wide range of cardiovascular effects which could all contribute to reduced mortality and improved health. The Division of Human Nutrition plans to conduct further research in this area on Australian DHA-rich oils. Oils rich in DHA would find ready markets when the greater potency has been demonstrated in well controlled laboratory and clinical trials.

There has been wide-spread interest in Australia in the importation of omega-3 products, but prior to the commencement of this FRDC project, there has been little development towards the production of an Australian-equivalent omega-3 oil or product. An objective of the project was to provide research to assist Australian industry to develop new marine oil-based products, and in the long term to 1. reduce the import of similar products from overseas, and 2. stimulate interest in export of Australian omega-3 products. The raw material is available and the potential now exists with the research capability and industry expertise available in Australia for products to be developed. With the acknowledged additional problem that fish oils from northern hemisphere waters may be contaminated by organochlorines and other deleterious materials, the potential for a "clean and green" Australian omega-3 product is further enhanced.

In this project, results were obtained for omega-3 PUFA composition for imported omega-3 containing encapsulated oils and for flesh and oils from selected Australian fish. The availability of such data is an important prerequisite for the possible production and marketing of home-grown omega-3 PUFA products by Australian industry.

Lipid composition and fatty acid composition of omega-3 capsule products

Commercially produced omega-3 PUFA capsules and fish oil capsules were purchased from local health food stores, supermarkets and pharmacies. The price, number of capsules, and the amount and value of PUFA varied between products (Table 2). The samples were divided into two groups of omega-3 products and one omega-6 product (Table 3). The first omega-3 PUFA containing-group was the cod-liver oil product. The remaining five brands comprised the second omega-3 group; all five samples had almost identical fatty acid compositions. Evening primrose oil, examined for comparative purposes, was the omega-6 PUFA-rich product.

Triacylglycerol was the only lipid class detected in all the capsules analysed. According to various researchers, the high degree of unsaturation in fish oil-based omega-3 capsules can cause oxidation of PUFA resulting in formation of polymeric materials such as dimeric, trimeric and oligomeric triacylglycerols. The oxidation products may play a role in carcinogenesis and inhibition of prostacyclin production as well as having other adverse biological effects. The absence of additional peaks in TLC-FID traces of commercial products analysed indicates the capsules are either recently manufactured or contain an antioxidant to prevent oxidation.

Cod-liver oil (CENOVIS) was the cheapest of all of the oils analysed and contained 16.7% saturated fatty acids, 47.7% monounsaturates ($C_{16:1}$, $C_{18:1}$, $C_{20:1}$ and $C_{22:1}$) and 33.5% PUFA (Table 3). The PUFA profile was dominated by DHA (11%), EPA (9%) and 20:4(n-3) (6%) and contained only minor amounts of the (n-6) PUFA. Although the CENOVIS product contains lower relative levels of PUFA than the other omega-3 PUFA products, consumption of seven capsules of the CENOVIS product (at approximately two thirds the price, Table 2) would supply a similar amount of omega-3 PUFA as one capsule of any of the other omega-3 products.

The remaining five omega-3 capsules were all encapsulated fish oils, which generally contained identical fatty acid compositions; these capsules were Fish Oil (Blackmores, Efamol), Omega-3 (Nature's Way), MaxEPA (Nature's Own, Vitaglow) (Table 3). PUFA were the dominant group of fatty acids (41%) with levels of monounsaturated [mainly 16:1(n-7)c and 18:1(n-9)c] and saturated (predominantly 16:0 and 14:0) fatty acids being slightly lower (26% and 29% respectively). The major PUFA in decreasing order of abundance were EPA (18%), DHA (11%), C_{16} PUFA (3%), 18:4(n-3)/18:3(n-6) (3%) and DPA (2%). The price of the different brands of omega-3 capsules in this subgroup varied between $235 and $298 per kg of oil at retail level.

All encapsulated fish oils contained a minimum of 30% omega-3 PUFA; evening primrose oil did not contain omega-3 fatty acids, but rather showed high levels of omega-6 fatty acids. With the exception of cod-liver oil which contained 11% DHA and 9% EPA, the other omega-3 capsules contained 11-12% DHA and 18% EPA.

Table 2. Listing of commercial encapsulated omega-3 and omega-6 (Evening Primrose oil) PUFA oils.

Brand	Name	No. capsules per jar	Price	Capsule mass (mg)	Price per capsule	Price kg oil	mg EPA per capsule	value per 100 mg EPA
Cenovis	Evening Primrose oil	50	$5.49	550	$0.11	$60.39	-	-
Cenovis	Cod-liver oil	90	$2.98	275	$0.03	$9.11	25	$0.12
Efamol (Vitaglow)	Fish oil capsules	40	$11.80	1000	$0.30	$295.00	180	$0.17
Nature's Own	MaxEPA Marine lipid concentrate	50	$11.75	1000	$0.24	$235.00	180	$0.13
Blackmores	Fish Oil 1000	50	$12.95	1000	$0.26	$259.00	180	$0.14
Vitaglow	MaxEPA PLUS	40	$13.50	800	$0.34	$270.00	115	$0.30
Nature's Way	OMEGA 3	60	$17.90	1000	$0.30	$298.33	180	$0.17

EPA, eicosapentaenoic acid

Table 3. Fatty acid composition of commercial encapsulated omega-3 and omega-6 PUFA oils[A].

Fatty Acid	*Percentage Composition*							
	CENOVIS Evening Primrose Oil	CENOVIS Cod Liver Oil	Blackmores Fish Oil	Efamol Fish Oil	Nature's Way Omega-3	Nature's Own MaxEPA	Vitaglow MaxEPA	Jack Mackerel Oil
14:1(n-5)c	-	0.1	-	-	-	-	-	-
14:0	-	4.1	8.2	7.6	7.6	8.1	7.6	7.2
i15:1	-	-	0.1	0.1	0.1	0.1	0.1	-
i15:0	-	0.2	0.2	0.2	0.3	0.2	0.2	0.1
a15:0	-	0.3	0.1	0.1	0.1	0.1	0.1	0.2
15:0	-	0.3	0.6	0.5	0.6	0.7	0.6	0.8
C16 PUFA	-	0.7	4.7	3.5	2.9	4.5	4.2	-
16:1	-	0.4	0.4	0.3	0.3	0.3	0.4	0.1
16:1(n-7)c	0.0	9.5	10.5	9.3	8.8	10.8	10.3	4.8
16:0	1.8	9.7	16.7	16.7	17.4	17.0	17.0	18.0
i17:1	-	0.3	0.2	0.3	0.3	0.2	0.3	-
i17:0	-	0.2	0.2	0.2	0.2	0.2	0.2	0.1
a17:0	-	0.6	0.3	0.3	0.3	0.3	0.3	0.4
17:0	0.0	0.1	0.5	0.4	0.4	0.5	0.5	0.6
18:4(n-3)/18:3(n-6) SA/GLA	4.4	2.9	3.2	3.2	3.7	3.1	2.9	2.7
18:3(n-3)	-	0.7	0.4	0.3	0.4	0.3	0.4	1.4
18:2(n-6) LA	91.2	2.1	1.5	1.3	1.6	1.3	3.2	1.8
18:1(n-9)c	1.6	16.0	8.4	8.9	8.6	8.3	8.9	10.8
18:1(n-7)c	0.2	4.2	3.2	3.3	3.1	3.2	3.5	3.2
18:1(n-5)c	-	0.4	0.1	0.2	0.3	0.1	0.1	0.2
18:0	0.5	1.9	3.2	2.6	2.6	3.1	3.1	3.8
20:5(n-3) EPA	-	8.8	17.9	19.5	17.8	17.9	17.5	9.8
20:4(n-3)	-	5.9	0.7	0.7	0.8	0.7	0.6	1.8
20:2(n-6)	-	0.2	0.2	0.2	0.2	0.2	0.2	-
20:1	-	11.5	1.4	1.5	1.8	1.5	1.6	10.4
20:1	-	0.3	0.3	1.2	1.0	0.3	0.2	0.4
20:0	-	-	0.2	0.2	0.2	0.2	0.2	0.1
22:6(n-3) DHA	-	11.0	11.3	11.1	11.1	10.9	10.6	11.0
22:4(n-6)	-	-	0.1	0.1	0.1	0.1	0.1	-
22:5(n-3) DPA	-	1.1	2.0	2.3	2.1	2.2	2.1	1.8
22:1	-	4.7	0.6	1.6	2.0	0.7	0.8	6.1
22:1	-	0.3	0.1	0.2	0.2	0.1	0.1	0.8
22:0	-	-	0.1	0.1	0.1	0.1	0.1	0.1
24:1	-	0.2	0.3	0.5	0.5	0.4	0.3	-
Other	0.2	1.2	2.1	1.5	2.5	2.4	1.5	1.1
Total	100	100	100	100	100	100	100	100
Sum saturates	2.3	16.2	29.5	28.1	28.8	29.6	29.1	30.6
Sum monounsaturates	1.8	47.7	25.2	27.0	26.7	25.6	26.4	36.8
Sum polyunsaturates	95.7	33.5	41.8	42.2	40.7	41.2	41.8	30.3
Sum Omega-3 PUFA	4.4	30.5	35.5	37.2	35.9	35.0	34.2	28.5

[A]All numbers are expressed as a percentage of the total fatty acids

Products are now available that are blends of fish oil and evening primrose oil. Evening primrose oil (CENOVIS) is derived from the evening primrose plant, native to North America. This oil is known to be rich in linoleic [LA, 18:2(n-6)]and γ-linolenic acid [GLA, 18:3(n-6)] and is currently cultivated in a number of countries due to its drought tolerance. The encapsulated evening primrose oil analysed in this study was abundant in LA (90%; 55% specification) and also contained minor levels of GLA (4%; 3.6% specification) (Table 3). This composition differs from the fatty acid composition of the plant oil alone (71% LA and 9% GLA). PUFA accounted for 95.7% of the total fatty acids.

Australian omega-3 fish oils

There is now increasing local interest in direct use of the lower value or unrefined marine oils for manufacture of new Australian products. Based on a predicted increasing market size and demand for omega-3 oils in nutritional products, the potential exists to add-value to the raw oils currently being manufactured locally. The Australian-derived oils available do not match the 18:12 ratio (weight percent) for EPA to DHA found in most encapsulated products (see Table 3). DHA is generally more abundant than EPA in Australian oils. The ratio of EPA to DHA varies considerably between species (e.g. Figure 4). 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 roles in human nutrition. Therefore precise knowledge of the relative levels of these two essential fatty acids will be important both when targeting oils for further development and as a marketing feature of future Australian products.

Australian species analysed, often on request, included the southern school shark, gummy shark, tiger shark and other shark species, banana prawn waste, ling, salmon (from several processes), bream, red bait, European carp (oil was obtained from several sources), spotted Wobbegong, jack mackerel, krill (several species) and cod liver oil (the latter for comparative purposes). Oil samples were derived from liver, waste material or whole fish. Results were usually prepared in report form and supplied to interested parties or prepared for scientific publication. A listing of reports and publications is provided in Appendix A and copies of non-confidential reports are available from the CSIRO Division of Marine Research library. Selected results are presented below.

Fish and related oils analysed which contain high levels of EPA and DHA include:

(i) oil produced from jack mackerel and from waste generated by the salmonid industry (under development by Groundswell Organics)
(ii) red bait (*Emmelichthys nitidus)* which is a by-catch associated with the jack mackerel fishery
(iii) mutton bird oil (Appendix D)
(iv) oil produced as a by-product from tuna canning (e.g. Heinz, Eden)
(v) oil derived from the southern ocean krill *Euphausia superba* and the local krill *Nyctiphanes australis* (Appendix D)
(vi) liver oil from school and gummy shark
(vii) Lutjanid species (snapper or sea perch)
(viii) oil isolated from selected trash fish associated with the northern prawn and orange roughy fisheries.

Results for these and other oils follow.

Oil from Atlantic salmon waste was examined several times during the project and shows a similar fatty acid profile to jack mackerel oil (Table 3). These oils contained reasonable amounts of the two essential PUFA EPA (10%) and DHA (11%). EPA and DHA levels were similar to levels detected in cod-liver oil. The cod-liver and jack mackerel oils both contained a similar level of DHA to the encapsulated commercial products. The main difference in the PUFA profiles was that the level of EPA in the jack mackerel and salmon waste oils (9-10%) was lower than determined for the imported commercial products (18%, Table 3). The current price of crude jack mackerel oil is between 50c and $1.00 per kg and this oil would require further treatment (e.g. removal of free fatty acids, decolourising and deodorizing) and packaging prior to marketing for applications other than incorporation in aquaculture feeds.

Liver oil from the southern school and gummy sharks contained elevated levels of the two essential fatty acids, EPA and in particular DHA (Figure 4). These results have been described in detail in Section 7.3. Based on the levels of PUFA present, these two oils and other oils of similar composition (e.g. ling liver oil) may offer potential for commercial use.

A systematic survey of six *Lutjanidae* species (snapper or sea perch) from northern waters was also performed. All species contained very high relative levels of the key omega-3 PUFA, DHA (Figure 4). These results are to be prepared for publication.

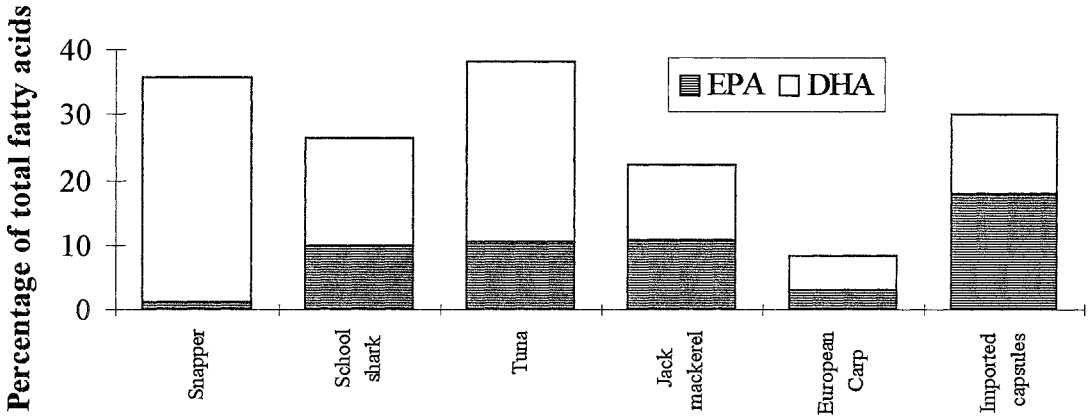


Figure 4. EPA and DHA content of selected oils from Australian species and imported fish oil capsules.

Selected trash fish (by-catch of existing fisheries) were examined during the project and a report was prepared. Oil from *Caelorinchus* sp. caught as by-catch of orange roughy contained 38% PUFA, with main components being DHA (20%), EPA (8%) and AA (3%). A trash fish caught abundantly during northern prawn trawling, *Lethrinus genevittatus*, also contained elevated PUFA (44%), with DHA dominant (32%). However, oil yield was low (3%) indicating little scope exists for utilization of this species for oil alone.

The major component of most of the oils described above is generally triacylglycerol, although the mutton bird (native dialect, Yolla), like the oreo dory oils can contain elevated levels of wax esters (e.g. mutton bird oil contains approximately 60% wax ester). Unlike the wax ester rich oils from orange roughy, dory and *Electrona* (section 7.2), mutton bird oil contained elevated levels of omega-3 PUFA (Appendix D).

Due to increased interest in environmental problems associated with increased numbers of European carp in Australian inland waters, the oil from this species was analysed. Results obtained were provided to several organizations and companies during the final year of the project. These data will aid considerations on possible uses for European carp. A summary follows.

Lipid and fatty acid composition of European carp oil

Lipid class composition

Four carp oil samples supplied by Victorian Inland Fisheries (two from fillets and two from guts/roe) showed similar lipid profiles: triacylglycerol (98-99.6%), polar lipid (0.4%), free fatty acid (0.2-0.6%), sterol (0.2-0.9%). No differences were observed between oil from the flesh and gut/roe. The low level of free fatty acids is indicative of the high quality of the oil; free fatty acids are present in oils of lower quality. In comparison, carp oil produced by Marchem showed triacylglycerols (71%), polar lipids (24%), free fatty acids (2%), sterol (2%) and an unidentified component (2%). In a separate analysis, the oil obtained by solvent extraction of fillets contained higher levels of polar lipid (88%). This finding indicates that processes used by Victorian Inland Fisheries and Marchem are not extracting the more polar lipid. The high triacylglycerol levels in carp oil produced by Victorian Inland Fisheries may be attractive to a market seeking oils for feed production or other forms of value-adding. European carp has a high oil content (i.e. 30-50% of mass, G. Darbyshire, personal communication). This is an attractive feature for commercial interest in obtaining carp oil either as a by-product of meal manufacture or from other processing.

Fatty acids

The fatty acid composition of the Victorian Inland Fisheries carp oil was comprised of mainly monounsaturated (56-57%) and saturated fatty acids (22-23%). PUFA were present at low relative levels (15-16%) (Table 4, Figure 5). The main fatty acids in carp oil were 16:0, 16:1(n-7)c, 18:1(n-9)c and 18:2(n-6), accounting for approximately 70% of the total fatty acids. Levels of the essential PUFA, EPA and DHA were low (<2% of each) compared to oil derived from marine fish. Higher levels of EPA and DHA (3% and 5% respectively) were observed in carp oil produced by Marchem. The oil obtained by solvent extraction of fillets contained higher levels of EPA (8.8%) and in particular DHA (33%) (Project 95/122). This finding again indicates that the processes used by Victorian Inland Fisheries and Marchem to obtain triacylglycerol-rich oils are not extracting the more polar lipid containing PUFA.

Fish oils are usually considered a good source of essential omega-3 PUFA. Fish oils containing high levels of monounsaturated fatty acids (e.g. orange roughy oil) are used in industrial degreasers and cosmetics. In orange roughy oil, the main constituent is wax ester, which has better solvating properties than triacylglycerol oils. Recently, increased interest has occurred in Australia with Emu oil. The carp oil fatty acid profile is similar to emu oil; both oils are mainly triacylglycerols, containing high levels of monounsaturates and low levels of PUFA.

It may be possible that industry will have uses for low-PUFA carp oil. Alternately, the levels of omega-3 PUFA present may be sufficient for use by some aquaculture operators, for incorporation into feeds and for other applications. Feeding trials with target species would be needed to further examine potential in this area. Fisheries management agencies regard the carp as a pest and strategies are being developed to remove it from Australian water-ways. Should harvesting of carp be considered, the results of this study may have wider interest.

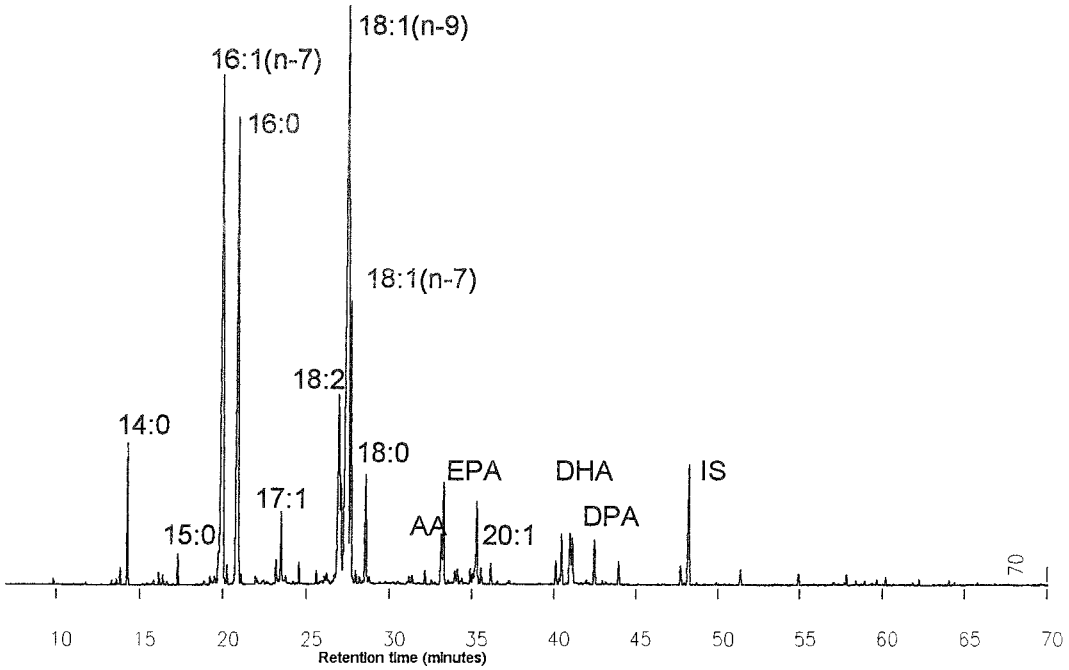


Figure 5: Partial gas chromatogram of fatty acids from European carp (supplied by Victorian Inland Fisheries). IS denotes internal standard.

Table 4: Fatty acid composition of carp oil (supplied by Victorian Inland Fisheries)

Sample	Percentage composition			
	Fillets		Gut/Roe	
	A1	A2	B1	B2
14:0	2.3	2.3	2.5	2.4
15:0	0.5	0.5	0.6	0.6
16:0	16.7	17.0	16.4	16.6
17:0	0.4	0.4	0.4	0.4
18:0	2.7	2.6	2.4	2.6
Sum Saturates	**22.8**	**23.1**	**22.4**	**22.8**
i17:0	0.6	0.6	0.6	0.7
a17:0 + 17:1(n-8)c	1.4	1.6	1.6	1.6
Sum Branched	2.4	2.6	2.8	2.8
16:1(n-9)c	0.7	0.0	0.0	0.0
16:1(n-7)c	18.2	19.5	20.1	19.4
16:1	0.3	0.3	0.4	0.3
18:1(n-9)c	28.1	28.3	28.6	28.3
18:1(n-7)c	5.7	5.6	5.3	5.1
18:1(n-5)c	0.2	0.2	0.2	0.2
20:1(n-9)c	2.1	2.1	2.2	2.5
20:1(n-7)c	0.4	0.4	0.4	0.4
Sum Monounsaturates	**57.0**	**56.7**	**57.4**	**56.6**
C16 PUFA	0.1	0.2	0.2	0.3
18:2(n-6)	7.5	8.2	8.5	8.7
18:3(n-3)	0.8	0.4	0.6	0.2
18:4(n-3)	0.1	0.1	0.2	0.3
20:2(n-6)	0.3	0.4	0.4	0.4
20:3(n-6)	0.3	0.3	0.3	0.3
20:4(n-3)	0.3	0.3	0.3	0.3
20:4(n-6) AA	1.4	1.5	1.6	1.5
20:5(n-3) EPA	1.7	1.8	1.8	1.7
22:4(n-6)	1.5	0.3	0.2	0.2
22:5(n-3) DPA	1.0	0.9	0.6	0.6
22:6(n-3) DHA	1.1	1.3	0.8	0.8
Sum PUFA	**16.2**	**15.8**	**15.7**	**15.5**
Other	2.1	2.4	2.3	2.8
Total	100.0	100.0	100.0	100.0

12:0, 20:0, 18:3(n-6), 22:1(n-9)c, 22:1(n-7)c also present (0.1-0.2%)

Commercial fisheries have recently been developed at Heard and Macquarie Islands for the sub-Antarctic Patagonian toothfish (*Dissostichus eleginoides*). In association with industry, the oil composition of this commercially important fish was examined. Samples of flesh, fish meal and rendered oil were obtained from the Austral Fisheries vessel "Austral Leader". The samples were from fish caught in waters adjacent to Macquarie Island in the Aurora trough 54° 35'S, 158°40'E at a depth of 700 meters between 16-20 February 1997. These results provide initial pertinent biochemical data for use by Australian industry to assist in maximizing the potential return from this sensitive fisheries resource at whatever catch levels are ultimately considered sustainable. Further analyses of samples collected from Heard Island and other regions, together with examination of the possible effect of diet, season and other environmental factors, is recommended. A summary of results supplied to industry in mid 1997 follows (see also Tables 5 and 6).

Lipid Composition of Patagonian toothfish (*Dissostichus eleginoides*) Flesh, Oil and Meal

The lipid and fatty acid composition of flesh, meal and rendered oil obtained from the waste tissue of Patagonian toothfish provided by Austral Fisheries were determined. All samples contained elevated levels of triacylglycerol (72-100% of total lipid). Oil content of the flesh was 1.8% (small fish) and 5.6% (large fish), with the meal containing 24% oil. The flesh and meal contained 14-23% PUFA. Based on the results from this study and earlier results obtained for the Patagonian toothfish (Final Report FRDC 91/77), fillets from this new Australian fishery represent a good source of the essential omega- 3 PUFA. In contrast, the oil produced by commercial rendering of waste material contained considerably lower levels of PUFA (6% of total fatty acids). The rendered oil contained higher levels of monounsaturated fatty acids (73%) than the other tissues (51-65%) and is similar in composition to oil derived from European carp.

Table 5. Lipid class composition of Patagonian toothfish.

Sample	Percentage composition					Oil content (mg/g)
	WE	TAG	FFA	ST	PL	
Meal	-	74	0.4	0.9	25	240
Flesh						
large fish	-	92	2	0.3	6	56
small fish	-	72	10	2	16	18
Oil	-	100	-	-	-	-

WE = wax ester, TAG = triacylglycerol, FFA = free fatty acid, ST = sterol, PL = polar lipid

Table 6. Fatty acid composition of Patagonian toothfish

Fatty acid	Percentage composition			
	Flesh-small	Flesh-large	meal	oil
14:0	3.6	5.1	3.1	5.0
15:0	0.4	0.4	0.4	0.5
16:1(n-9)	0.6	1.4	0.6	0.4
16:1(n-7)	7.7	8.8	7.4	10.3
16:1(n-5)	0.3	0.3	0.2	0.2
16:0	17.5	17.7	14.8	15.7
a17:0/17:1	0.1	0.1	0.0	0.0
17:0	0.3	0.3	0.3	0.2
18:4(n-3)	0.0	0.1	0.0	0.0
18:2(n-6)/18:3(n-3)	0.0	0.0	1.6	1.7
18:1(n-9)	25.4	31.1	33.3	40.3
18:1(n-7)	5.4	4.7	6.1	6.3
18:0	3.5	3.8	3.6	0.0
20:4(n-6) AA	0.0	0.0	1.2	0.3
20:5(n-3) EPA	7.4	5.9	4.0	1.8
20:3(n-6)	0.2	0.1	0.1	0.0
20:4(n-3)	0.0	0.0	0.3	0.3
20:2(n-6)	0.0	0.2	0.3	0.2
20:1(n-9)/(n-11)	8.3	6.9	9.5	9.2
20:0	0.0	0.1	0.1	0.1
22:5(n-6)	0.0	0.1	0.0	0.0
22:6(n-3) DHA	14.5	8.8	6.1	1.4
22:5(n-3)	0.6	0.4	0.3	0.2
22:4(n-3)	0.1	0.1	0.0	0.0
22:1(n-11)	2.4	1.8	3.3	3.2
22:1(n-9)	1.1	1.0	1.8	1.8
22:1(n-7)	0.2	0.2	0.3	0.2
22:0	0.2	0.1	0.0	0.0
24:1	0.5	0.4	1.0	0.6
Total	100.0	100.0	100.0	100.0
Sum saturated	25.5	27.5	22.3	21.5
Sum monounsaturated	51.5	56.6	64.7	72.6
Sum branched	0.1	0.1	0.0	0.0
Sum polyunsaturated	22.8	15.8	14.0	5.9
20:5(n-3) EPA (mg/100 g)	46	86	495	ND
22:6(n-3) DHA (mg/100 g)	89	127	765	ND

ND, not determined

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 the processing required to produce competitive products. Such processing could include: separation of unwanted classes using degumming and filtration, enrichment of omega-3 PUFA using "winterizing" techniques or through treatment with lipases and re-synthesis of triacylglycerols, decolourizing and deodorizing, addition of other components (e.g. antioxidants/vitamins), encapsulation, etc. Several of these steps may be required to produce a higher value-added product.

The blending of oils and/or fractions of oils is now being performed by industry and will increasingly occur. For example, Clover Corporation recently has blended an imported DHA-containing tuna oil with evening primrose oil (ratio 4:1) to manufacture products marketed as "Milkarra" and "Karicare" for use in infant formulas (Clover Corporation, personal communication). Clover submitted the product to the TGA in 1995 and have since completed the necessary trials and related documentation. This FRDC project provided key analytical support to the company during product development and the subsequent approval process. When an Australian-produced omega-3 oil becomes available, the company's intention is to replace the imported oil with the local value-added oil. Further research was performed on laboratory purification of Australian tuna oil and is described in section 7.8.

The CSIRO Division of Human Nutrition has recently indicated that DHA may be more active (than EPA) against a range of cardiovascular disorders. The composition of the Australian marine-derived oils may therefore be considered advantageous for future product development. The first animal cardiovascular trials of an Australian omega-3 oil product (derived from tuna oil) was conducted by the CSIRO Division of Human Nutrition. It would be useful and potentially beneficial to conduct similar trials with other purified Australian omega-3 oils (and also eventually with enrichment products from jack mackerel, salmon and other oils).

The possible requirement of larger quantities of fish oil by the expanding Australian aquaculture industry may be in direct competition to other industries needing sources of omega-3 PUFA-rich oils for value-adding. Further consideration of this issue will be needed in the future.

In performing the research described above and examining the literature available on the PUFA (and lipid class and cholesterol) content of Australian species, a general lack of high quality data for many species was noted. The FRDC project (95/122) "Nutritional value of Australian fishes: oil, fatty acid and cholesterol composition of edible species" was initiated and commenced in late 1995. This new project compliments the FRDC-funded project: "Handbook of Australian Seafood - A Guide to Whole Fish and Fillets" and in addition will build on research conducted in Project 94/115.

A pilot experiment was conducted with scientists from the University of Tasmania on the use of novel omega-3 PUFA containing bacteria as feed for rotifers that are used as larval feed in aquaculture. A manuscript was published in Aquaculture on this initial research. (Appendix B). A summary of the findings follows and a poster detailing the discovery of specific bacteria as a source of omega-3 PUFA is shown in Appendix C. Following the success of this initial research, and industry interest, a new project to evaluate the use of these bacteria in aquaculture and other arenas was initiated. This project is now the subject of a separate FRDC study (97/329; T. Lewis *et al.*).

Enrichment of the rotifer *Brachionus plicatilis* fed an Antarctic bacterium containing polyunsaturated fatty acids

The Antarctic bacterium, strain ACAM 456, is known to produce eicosapentaenoic acid [20:5(n-3), EPA]. Following growth in batch culture, suspensions of this bacterium, at initial concentrations of 10^7, 10^8 and 10^9 cells ml^{-1}, were used as foods for three respective cultures of the rotifer *Brachionus plicatilis*. At 6 and 24 h, rotifers were removed, harvested and extracted for analysis of fatty acid composition, which was compared to that of rotifers grown on baker's yeast. Incorporation of EPA, along with bacterial fatty acid markers (i13:0, i15:0 and 14:0), was evident at all bacterial food concentrations tested. The highest observed incorporation occurred when rotifers were grown in the medium initially containing 10^9 bacteria ml^{-1}. After 24 h of feeding, the level of EPA reached 9.4% of total fatty acids in the fed rotifers (6.7 ng of EPA $rotifer^{-1}$). ACAM 456, a bacterial strain with the ability to produce EPA, was therefore shown to be a potential alternative enrichment food for the rotifer *Brachionus plicatilis* under feeding conditions that may be applicable to many Australian aquaculture operations.

A survey of selected southern ocean fish and zooplankton species was completed in collaboration with Prof C. F. Phleger from San Diego State University. Results for *Electrona antarctica* and *Clione limacina* are shown in sections 7.2 and 7.6. Results obtained for other zooplankton have been prepared for publication in the international literature (Appendix D) and a summary follows.

Lipids and Trophodynamics of Antarctic Zooplankton

Zooplankton were collected by trawl from the Elephant Island region of the Antarctic Peninsula, and from East Antarctica near 63 - 65 S and 139 - 150 W. Most zooplankton had low percentages of wax esters (0-8%, as percent of total lipid), with the exception of *Thysanoessa macrura* (34% wax esters). High triacylglycerol levels were found in *Themisto gaudichaudii* (68%, as percent of total lipid), *Euphausia tricantha*, and *E. frigida* (27-54% triacylglycerol), and *Periphylla periphylla* (42-48% triacylglycerol). Polyunsaturated fatty acids (PUFA) were 23-60% of the total fatty acids, with the omega-3 fatty acids eicosapentaenoic acid [20:5(n-3)] and docosahexaenoic acid [22:6(n-3)] being most abundant. The scyphomedusan, *P. periphylla*, was an exception with 12 - 19% docosapentaenoic acid [22:5(n-3)] being the major PUFA. The major euphausiid sterols included cholesterol (75-92%, as percent of total sterols) and desmosterol (6-22%). The major sterols of other zooplankton species were more diverse and included trans-dehydrocholesterol, 24-methylenecholesterol, brassicasterol, and 24-nordehydrocholesterol. The benthic ascidian, *Distaplia cylindrica*, had 45% stanols, as percent of total sterols, whereas the pelagic ascidian *Salpa thompsonii* had only 8-11% stanols. Trophodynamic implications of the lipid, fatty acid, and sterol data include an ability to distinguish herbivorous and carnivorous diets and determine survival and reproductive strategies.

7.8 Pilot examination of existing and new technologies (including SFE) for enriching shark liver oils and omega-3 PUFA from fish oils.

Squalene and squalane

The developing Australian industry has now used several technologies to separate and purify squalene from shark liver oils. In addition to research on squalene, we examined the potential to produce squalane (saturated counterpart of squalene) for both the domestic and international markets. During year 2, a large portion of project time was devoted to refinement at laboratory scale of a process to produce squalane. Scaling-up of the process was undertaken, both at a larger laboratory scale and in the pilot facility at the Division of Molecular Science (formerly Chemicals and Polymers). Pure squalane was successfully produced from a number of raw oils (containing between 40-80% squalene) supplied by industry. The technology was not patented, rather a report was completed on the research which has been maintained as confidential know-how with negotiations presently underway with interested parties. A summary follows.

CSIRO process for recovery of squalane from shark liver oil

An environmentally benign method has been developed for producing high purity (>99%) squalane from liver oils of deep-sea sharks (Figure 6). Industrial applications for the compound include cosmetics, perfumeries, lubrication, or as a high-boiling solvent. Yields in the order of 90% are obtained routinely by the process, which has been optimized and demonstrated on a multi-kilogram scale in a pilot plant.

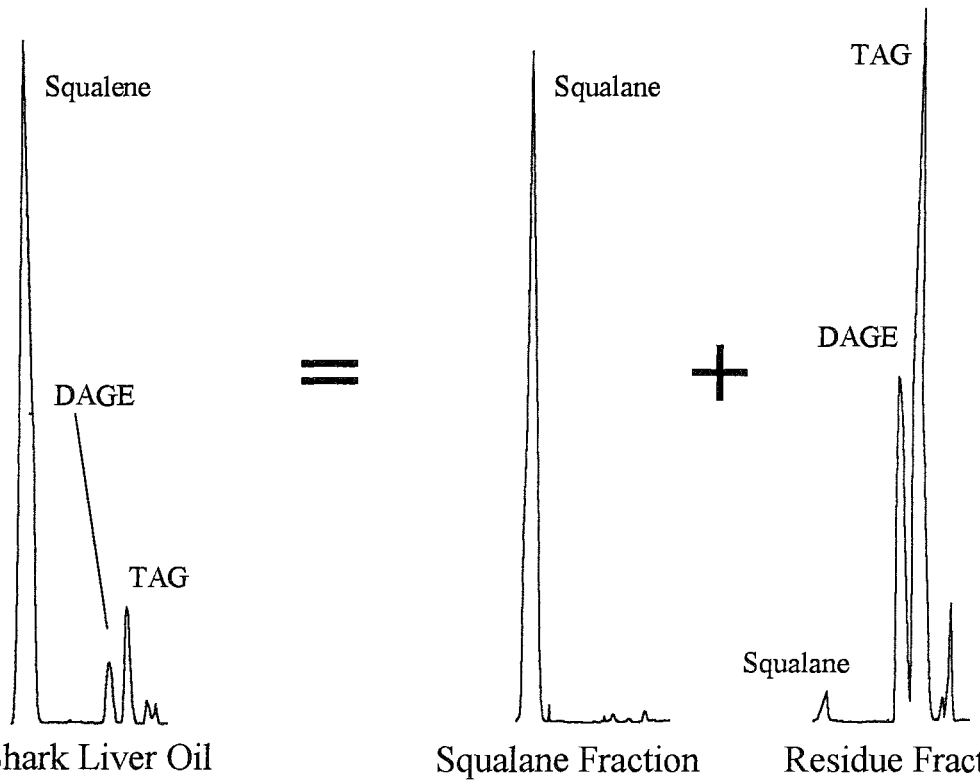


Figure 6. TLC-FID traces illustrating lipid class composition of raw shark liver oil and separated products after processing to produce squalane. TAG, triacylglycerol; DAGE, diacylglycerol ether.

In addition to optimizing squalane recovery and purity, the production and isolation of a saturated DAGE and TAG by-product (Figure 6), was also examined. The potential use of the saturated DAGE and TAG by-product needs to be investigated (e.g. in aquaculture feeds). Initial liaison within the Division's aquaculture program has commenced.

With the CSIRO Division of Molecular Science and industry, further development of a laboratory process to purify omega-3 PUFA fish oils was completed (Figure 7). The process produces purified triacylglycerol oils (>99%) which may be used in food, nutraceuticals and other products. The process was successfully scaled up to a 3-5 kg scale and has been subsequently licensed to Nu-Mega Lipids, a joint venture between Clover Corporation and Heinz (section 7.9). Further scaling up of the technology was also performed with Clover Corporation during late 1996 and 1997, with production trials scheduled for late 1997. Reports were prepared on research conducted during the scale-up of the process (Appendix A). A copy of a poster showing results on the oil composition of tuna species (Project 95/122) and the development of Australian tuna oil products (Project 94/115) is shown in Appendix C. The poster was presented in November 1997 at the Pacific Oils Conference 2000 in Auckland.

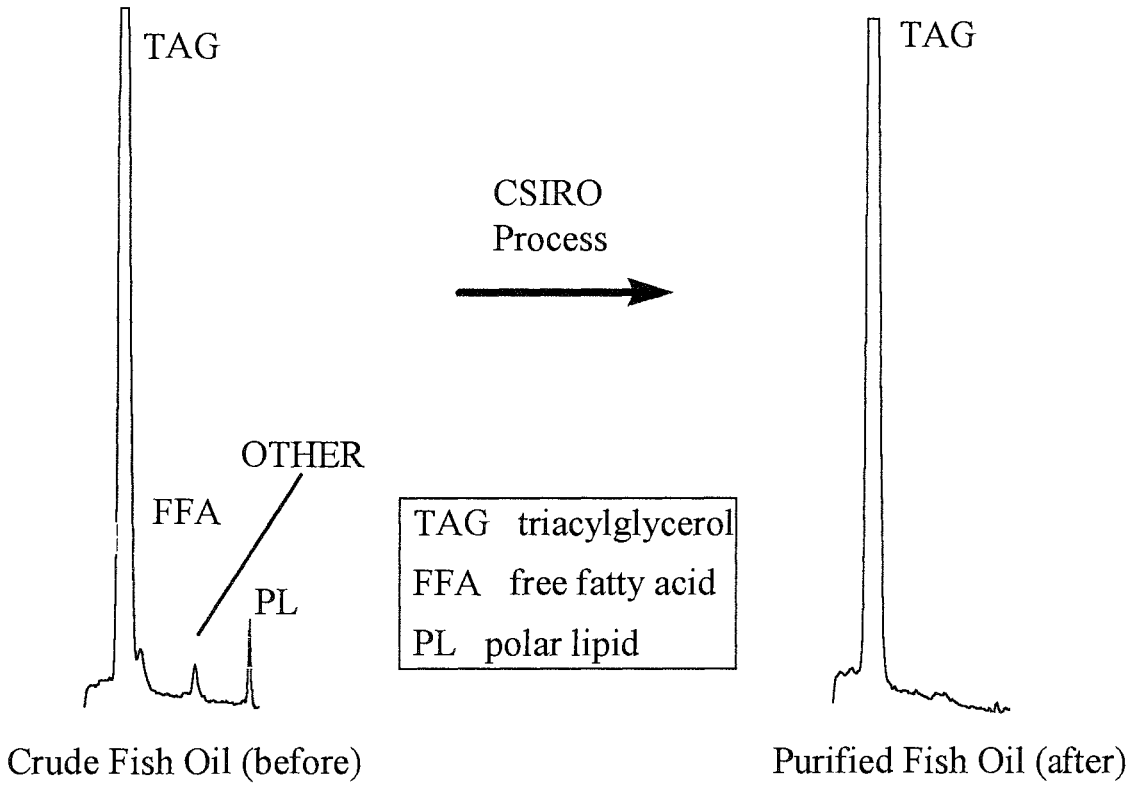


Figure 7. TLC-FID traces illustrating lipid class composition of crude tuna oil and purified product.

A proposal for the "Cardiovascular evaluation of Australian DHA-rich fish oil" was submitted to the FRDC in late 1995 by the CSIRO Division of Human Nutrition. It was planned that the oil to be used in the proposed trials would be produced by the Divisions of Marine Research and Molecular Science using the technology developed during this current project. The proposal was not funded and rather FRDC undertook to fast track collation of information relating to the health benefits of the consumption of Australian fish and fish products. Results from our project and project 95/122 were made available to the FRDC-appointed consultant during this process.

Supercritical fluid extraction (SFE) equipment was trialled for extracting and enriching shark liver oils. An Hewlett Packard SFE unit was trialled at the CSIRO Marine Laboratories. A report was prepared detailing the results for SFE purification of both omega-3 PUFA and shark liver oils (see Appendix A). A summary of results follows.

Use of Supercritical fluid extraction for enriching marine oils: Enrichment of squalene and triacylglycerols

Squalene

Three shark liver oils of varying composition (47%, 64% and 87% squalene) were tested to determine whether SFE could be used to enrich squalene. Squalene of 99% purity was obtained by SFE extraction of all three oils, with yields varying between 94% to 100%. The use of SFE also enriched squalene to 99% purity from oils where the squalene levels (40% to 50%) are generally considered too low for processing by other means. The SFE products were odorless, suggesting that the process may have advantages over other technologies. Other components (e.g. DAGE and triacylglycerols) were not detected in the final extracts and based on their solubility in CO_2, it was concluded that the use of hexane as the rinse solvent did not remove the components from the alumina trap packing. The selective collection of other components from crude shark liver oil will require further investigation. It is also presently unclear, in the case of the endeavour dog shark liver oil, whether or not pristane was removed during the extraction process. If pursued, future research should also address this aspect of the purification/enrichment procedure.

Triacylglycerols

After determining the optimum extraction conditions for a standard purified TAG-rich fish oil, crude oils were extracted to optimize extraction conditions. Crude tuna oil (91% TAG) previously purified to remove FFA was trialled to determine if the SFE would remove wax ester, sterol, polar lipid and unknowns. SFE partly removed wax ester and sterol from the crude oil while polar lipid and the unknown were fully removed; a TAG purity of >99% was achieved. A crude tuna oil was trialled to determine whether FFA could also be removed. FFA decreased from 1.8% to not detectable and polar lipid was also totally removed, while the level of sterol remained at trace levels (0.3%). TAG was enriched from 95.6% to 99.7%. In order to determine whether sterol could be removed, prawn waste oil (high sterol content) was extracted. The crude prawn oil contained 10% TAG, 26% FFA, 10% sterol and 54% polar lipid. Two fractions were obtained by SFE. The first fraction contained 95% TAG, 1.5% FFA, 2.6% sterol, 0.1% polar lipid and 0.7% of an unknown component. The methanol fraction contained 90.7% FFA, 0.3% sterol and 8.7% polar lipid. SFE was effective for removing FFA, sterol, polar lipid and some unknowns. It is unclear whether removal is due to their "poor extractability" by CO_2 or if selective solvent rinsing and trap packing allowed only the desired components to be collected. Further studies should determine if such a process would be feasible on a larger scale.

Fatty acid profiles of triacylglycerol-containing oils purified by SFE

The fatty acid composition of selected oils was determined to assess if SFE purification of TAG enhanced PUFA levels. In the case of tuna oil pre-processed to removed FFA, levels of the essential PUFA, EPA and DHA, were enhanced. Levels of EPA increased from 3.5% to 4.7% and DHA increased from 20.2% to 28.3%, indicating that SFE may be an effective tool for enriching these components.

In conclusion, the results of analytical-scale SFE trials indicate the approach offers an alternate method to enrich marine oils, or to supplement existing technologies. Oils produced were of high quality with respect to purity and odor. Future investigations could also address if such purified oils can be obtained directly from raw materials (e.g. livers). Enrichment of PUFA was possible, although other researchers have found that for separation by SFE, the fatty acids need to be present as derivatives (e.g. esters). Further research is needed to verify this finding.

PUFA Enrichment

The current imported omega-3 products typically contain 25-40% EPA and DHA. Enrichment of the oil can result in EPA and DHA levels being increased to 50-60% or greater. Research was conducted on the development of technology for enriching Australian omega-3 oils. This research also was performed collaboratively with the CSIRO Division of Molecular Science. Phase I of the research resulted in the development of a purification regime for refining crude tuna oil to produce fractions rich in omega-3 PUFA. A report was completed describing the process (Appendix A). A summary of results follows.

Process for enrichment of fish oil-derived polyunsaturated fatty acids

A purification regime for the refining of crude tuna oil to produce fractions rich in omega-3 PUFA was developed. The regime included esterification of the TAG at a yield of >90%, followed by treatment of the ester mixture to separate the PUFA esters. The yield of this second step was approximately 20%. The combined EPA and DHA level was approximately 95% of the total fatty acids (Figure 8). A low pressure fractionation of the urea-free supernatant yielded individual PUFA ethyl esters, with DHA of greater than 90% purity achieved. Hydrolysis then afforded the free PUFA. It is envisaged that by-products of the regime will be of use in lower-value products.

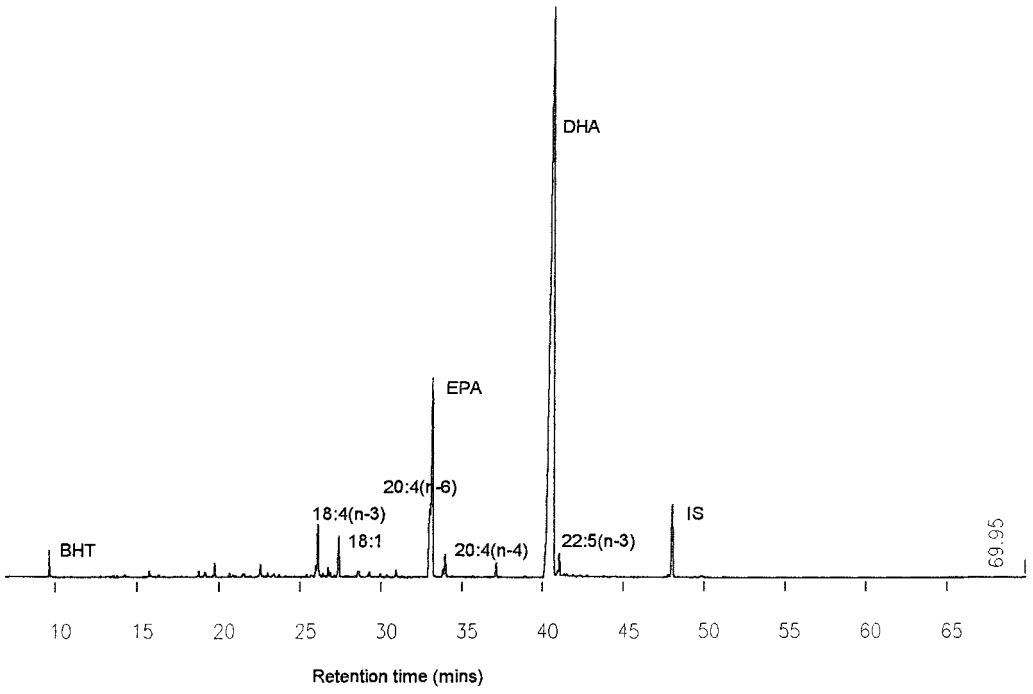


Figure 8. Partial gas chromatogram of fatty acids from enriched tuna oil. IS denotes internal standard.

It will be possible to use the enrichment process on either free fatty acids or fatty acid esters (e.g. ethyl esters) derived from tuna or other omega-3 containing oils. In analysis of a Japanese DHA product provided by industry, it was noted that the product was in triacylglycerol form. Other products available and examined during the project contained PUFA in ester or free acid form. Should the market require triacylglycerol rather than free acid or ester omega-3 enriched products, it will be necessary to re-synthesize triacylglycerols from the enriched fatty acid or ester products and glycerol.

The bioavailability of the omega-3 fatty acids is dependent on the form of the fatty acid (triacylglycerol, free acid, ester). Encapsulated omega-3 oils have traditionally been comprised of the intact triacylglycerol, which are also more stable than the free acids and esters. Enrichment procedures generally are more easily performed on free acids or esters, therefore the marketing of these forms of omega-3 products over the triacylglycerol form may have some advantages if a viable market can be identified for these products. During the final stages of the project, negotiations were commenced to provide the PUFA-enrichment technology under an options agreement to potential commercial partners. Appropriate patent searching was completed.

Aspects of the PUFA enrichment results were presented at the Inaugural workshop of the Australasian Section of the American Oil Chemists' Society (Canberra, February 1997). An additional oral presentation was given on behalf of FRDC describing the Corporation and providing examples of R&D on marine oils supported by FRDC. The papers for the two AAOCS presentations are attached (Appendix D). Two talks highlighting developments with omega-3 PUFA oils in Australia were also presented at international meetings in France and Italy in September 1997. A manuscript submitted at the French meeting is attached (Appendix D).

7.9 Achievements of project for Australian industry

The oil composition has been determined for the first time for a number of species landed in Australian waters. Results were communicated directly to industry in report or other suitable form (Appendix A). The close ties established with Australian companies as part of an earlier FRDC-funded project (91/77) continued, enabling the project to interact strongly with industry. During the project companies and organizations that have sought or been provided with advice, literature or detailed analyses on oils being produced in Australia include:
Agrivet, Albert Park Seafoods, Antarctic Division, Antarctic CRC, ATG, Austral Fisheries, BEKU Environmental Products Ltd, Bergilden, Biodeva, Clipper Pearls, Clover Corporation, CSIRO Fisheries (prior to 1997 merger), CSIRO Human Nutrition, CSIRO Leather Research Centre, CSIRO Plant Industries, Eden Consulting, Foxbay, Gibsons, Gibson Chemicals, Goulburn Valley Authority, Groundswell Organics, Heng Yin Enterprices Development Co (Australia), HRL Technology, ICI, IFIQ, ITL, Jurox, MacFarlane Laboratories, Marchem, Marine Biologics, Murray Darling Association Inc., National Carp Task Force, Novost Pty Ltd, Nu-Mega Lipids, NZ Crop and Food Research, NZ Pharmaceuticals, Organic Solutions, RMIT, RP Scherer, Scales, Scandinavian Laboratories, Shaun Somerset (for FRDC), Solutions in Agriculture, Squalus, Tallay's Fisheries, Tasmanian Wakame, Tassal, Tas Univalve, Traicon Holdings, University of Tasmania (Agricultural Science, Aquaculture, Biochemistry, Chemistry, and other departments), Wallace Brothers and Zootech.

An article was prepared by a freelance reporter for ECOS, CSIRO's quarterly Science and

Environment magazine. Input for the article was provided by project scientists. A copy of the article is attached (Appendix D). The article has been provided to personnel from many of the above groups and companies.

As noted in section 7.8, a process was jointly developed by the CSIRO Divisions of Marine Research and Molecular Science to purify omega-3 PUFA oils. It is envisaged that the process could be used to add-value to crude oils currently produced in Australia. A provisional patent titled "Purification Process" was lodged in January 1995 and refiled in 1996 and 1997. Negotiations proceeded and a research agreement was completed with Nu-Mega Lipids in 1997 for the further development and licensing of the process for purifying omega-3 PUFA oils produced in Australia. Application of the technology is presently planned for developing value-added products from tuna oil. Research during late 1996 and 1997 centered on scale-up of the process and production of the first Australian-made purified omega-3 oil is scheduled for late 1997.

During the project, ongoing oil characterization, process optimization and development of plant protocols was performed in association with industry on a by-product fraction obtained during squalene production. The fraction is rich in DAGE (the term alkoxyglycerol is also used), which have perceived benefits against various disorders. Several reports were prepared on this oil fraction, including the detailed analysis of this fraction. A new Australian export product was launched by BEKU Environmental Products in late 1997 (Section 7.6, Appendix E).

An agreement was finalized between CSIRO and Squalus for the company to separate squalene from shark liver oils using a process developed by CSIRO during the previous FRDC marine oils project (91/77). It is envisaged that for transfer, to Australian industry, of the squalane technology developed and optimized at laboratory scale, a similar approach will be taken to that used with the squalene technology, i.e. a licensing agreement with a commercial partner will be sought. At this stage, industry is examining the process.

8. BENEFITS

The principal benefactors of Project 94/115 have been and will continue to be the individual companies attempting to exploit the marine oil resources associated with the by-products and by-catch from the Australian fishing industry. The fishing industry itself will benefit from new markets for by-products and by-catch. The community at large will benefit through the more efficient usage of by-products and by-catch, 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 imported materials. The Australian marine oils industry is still young and fragmented, with many individual players. As such, strong support for this project has been provided by individual companies within the industry rather than by management bodies for the Marine Oils or fishing industries.

Benefits also include potential better exploitation of environmentally sensitive species, such as Patagonian toothfish and European carp. Results for these two species have been supplied to management authorities and industry and will aid future decisions to be made with these fisheries. Additional benefits were the initiation of new projects that will examine the lipid composition of Australian Seafood (Project 95/122) and assess the potential of using novel PUFA-containing

bacteria in aquaculture (Project 97/329). These projects have already attracted positive media and other interest for FRDC and the collaborating institutes.

9. INTELLECTUAL PROPERTY

The intellectual property generated during the project includes detailed oil compositional data on many fish-derived and related oils. Much of these data have been published or provided to clients in report form.

In addition, oil processing technology has been developed for the isolation of squalene (and associated by-products), the isolation of squalane (and by-products), purification of marine TAG and enrichment of PUFA. The technology developed has been documented in report form. Several of these processes have had provisional patents lodged and refiled, whilst others have been maintained as confidential reports that can be made available to prospective industry partners.

10. FURTHER DEVELOPMENT

Research undertaken with FRDC project 94/115 (and the preceding Project, 91/77) has provided further impetus and assistance to the relatively new Australian Marine Oils industry, with an emphasis on first, orange roughy and related oils, secondly on shark liver oils and thirdly on omega-3 PUFA containing oils. Ongoing research is needed to enable the small businesses involved in the Marine Oils industry to continue to be viable. For Australian industry to achieve an economically sustainable level of value-added products, there will be a need to strengthen our knowledge of the oil composition of further new commercial and by-catch 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 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. Understanding the potential of Australia's natural and processed marine oils is important for both human nutrition and many primary industries such as aquaculture. In the long term the increased knowledge and technology base on marine oils and products will ensure more efficient return on the exploitation of Australian marine resources.

Highly-purified orange roughy and related oils may be used as a possible substitute for jojoba oil. These oils are utilized by the cosmetic and pharmaceutical industries after being refined, depolarized and deodorized. The further development and scale-up of technology for purification of wax ester oils in Australia is needed.

Future research and development is required to meet the needs of the Australian Marine Oils industry and includes:

- Continued determination of the composition of marine oils from underexploited pelagic and

other species, with particular reference to:
(i) identifying further optimum sources of omega-3 polyunsaturated fatty acids and other specialty chemicals (e.g. diacylglycerol ethers from deep-sea and other sharks and other sources), and
(ii) further assisting industry to develop products from these oils and specific oil fractions.

- Further examination, optimization and scale up of methods for obtaining omega-3 containing marine oils, thereby increasing product value. For example, production of purified individual PUFA oils (as esters or free acids) has been demonstrated during this project and research could be continued on these or other processes. The techniques developed should be broad-based and transferable between fish oils (and/or krill) and the developing field of single cell oils (SCO) derived from microorganisms.
- Cloning of PUFA gene fragments from bacteria (or other heterotrophs and possibly algae) into known organisms with an overall objective of developing new mechanisms for the synthesis and accumulation of oils rich in PUFA.

11. STAFF

Dr. Peter Nichols	Prin. Res. Sci.	BSc (Hons), PhD	40%
Dr. Nick Elliott	Sen. Exp. Sci.	BSc (Hons), PhD	5%
Mr. Michael Bakes	Exp. Sci.	BAppSc (Hons)	100%
Mr. Ben Mooney	Exp. Sci.	BSc (Hons)	20%

12. ACKNOWLEDGMENT

This project benefited from close collaboration with the CSIRO Division of Molecular Science (formerly Chemicals and Polymers) led by Chris Strauss and the strong interest provided by Australian industry, in particular from BEKU Environmental Products and Clover Corporation. Chris Fandry provided the support and leadership to the project that allowed marine oils research to commence at CSIRO and to develop strong ties with industry. Other CSIRO colleagues are thanked for ongoing support during the project: Michael Bessell, graphics; Danny Holdsworth, GC-MS operation; Greg Simpson, Teresa Cabelski, Michael Faulkiner and Stuart Littler, CSIRO Process Bay support; Mina Augerinos, technical assistance; Toni Cracknell,word processing. Colleagues from the Australian Collection of Antarctic Microorganisms, particularly David Nichols, and Rick Phleger and Brad Wetherbee collaborated with the Marine Products group during the project and are kindly thanked. Hewlett Packard supplied the SFE apparatus used during the project.

13. APPENDICES

A. Publication, reports, conference presentations and patents
B. Risk Assessment and Consideration of the International Environment. Report prepared for FRDC at completion of year 1 of Project 94/115
C. Marine Oils Poster presented at AMSA, Australasian AOCS Workshop, AOCS, Pacific Oils 2000 and other conferences
D. Scientific papers
E. Selected letters of support from clients to Marine Oils project
F. Selected media articles

APPENDIX A

Publications

Bakes, M. J. and Nichols, P. D. (1995) Lipid, fatty acid and squalene composition of liver oil from six species of deep-sea sharks collected in southern Australian waters. **Comp. Biochem. Physiol. 110B:** 267-275).

Woodward, D. R., Riley, M., Buick, D., Nichols, P. D. and Nichols, D. S. (1995) Nutritional analysis of the flesh and oil of Yolla, the Tasmanian mutton bird *Puffinus tenuirostris*: a useful source of omega-3 fatty acids. **Australian J. Nutrition and Dietetics. 52**: 8791.

Bakes, M. J., Elliott, N. G., Green, G. J. and Nichols, P. (1995) Within and between species variation in lipid composition of Australian and North Atlantic oreo and orange roughy: regional specific differences. **Comp. Biochem. Physiol. 111B**: 633-642.

Bakes, M. J., Nichols, P. D. and Strauss, C. R. (1995) Value-added products from Australian shark liver oils. In: **Recent Advances in Marine Science and Technology**. Proceedings PACON 1994 Conference (O. Bellwood, H. Choat and N. Saxena, Eds.) pp. 47-53, Townsville, July 1994, James Cook University of North Queensland.

Virtue, P. Johannes, R. E., Nichols, P. D. and Young, J. W. (1995) Biochemical composition of *Nyctiphanes australis* and its possible use as an aquaculture feed source: lipids, pigments and fluoride content. **Marine Biology 122:**121-128.

Mancuso, C. A., McMeekin, T. A., Nichols, D. S. and Nichols, P. D. (1995) The Australian Collection of Antarctic Microorganisms (ACAM): an increasing demand for a new resource. **Chemistry in Australia 62(8):**11-12.

Nichols, D. S., Hart, P., Nichols, P. D. and McMeekin, T. A. (1997) Enrichment of the rotifer *Brachionus plicatilis* fed an Antarctic bacterium containing polyunsaturated fatty acids. **Aquaculture 147**: 115-125.

Nichols, D. S., Nichols, P. D., McMeekin, T. A. and Sullivan C. W. (1996) Polyunsaturated fatty acids, and a novel C31:9 polyene, from sea ice microbial communities. In: Preprints of Papers Presented at 212th American Chemical Society National Meeting, Orlando, FL, vol. 36(2), Division of Environmental Chemistry, Inc., American Chemical Society, pp. 283-285.

Nichols, D. S., Brown, J., Nichols, P. and McMeekin, T. A. (1997) Production of eicosapentaenoic and arachidonic acids by an Antarctic bacterium: Response to growth temperature. **FEMS Letters** (in press).

Phleger, C. F., Nichols, P. D. and Virtue, P. (1997) High levels of wax ester in the Myctophid fish *Electrona antarcticum*. **Antarctic Science**. 9:258-265.

Phleger, C. F., Nichols, P. D. and Virtue, P. (1997) Southern ocean pteropods: a potential buoyancy role for diacylglycerol ether. **Lipids**. 32: 1093-1100.

Nichols, P. D., Bakes, M. J. and Elliott, N. G. Docosahexaenoic acid-rich liver oils from

temperate Australian sharks. **Marine and Freshwater Research.** (manuscript submitted).

An article was prepared by a freelance reporter for ECOS, CSIRO's quarterly Science and Environment magazine. Input for the article was provided by project scientists.

Good oils and fishy tales. (1996) **ECOS** 87: 12-15.

Reports *(held at CSIRO Marine Laboratories)*

Bakes, M. J. and Nichols, P. D. (1994) Lipid and fatty acid composition of liver oil isolated from southern school shark (*Galeorhinus galeus*). Report 94-SC2. Report prepared for Scales.

Bakes, M. J. and Nichols, P. D. (1994) Assay of lipid classes in Orange roughy oil produced by Quotila Trading. Report 94-QT1. Report prepared for Quotila Trading.

Nichols, P. D. and Bakes, M. J. (1994) GC assay of BEKU sualene fractions. Report 94-BEKU5. Report prepared for BEKU Environmental Products Ltd.

Bakes, M. B. and Nichols, P. D. (1994) A study of the lipid and fatty acid composition of the oil extracted from banana prawn waste. Report 94-AVR1. Report prepared for Agricultural Vetinary Research.

Bakes, M. J. and Nichols, P. D. (1994) Lipid composition of shark liver oils supplied by Scales. Report 94-SC4. Report prepared for Scales.

Bakes, M. J. and Nichols, P. D. (1994) Proximate lipid composition, squalene content and fatty acid composition of a New Zealand shark liver oil. Report 94-C&FR3. Report prepared for the Crop and Food Research Institute.

Bakes, M. J. and Nichols, P. D. (1994) Lipid and fatty acid composition of veterinary-grade cod liver oil. Report 94-Zootech1. Report prepared for Zootech.

Bakes, M. J. and Nichols, P. D. (1994) Lipid and fatty acid composition of liver oil isolated from ling and three species of shark. Report 94-SC3. Report prepared for Scales.

Bakes, M. J. and Nichols, P. D. (1994) Assay of lipid classes in northern Australian shark liver oils supplied by Scales. Report 94-SC6. Report prepared for Scales.

Nichols, P., Bakes, M. and Elliott, N. (1995) Marine oils from Australian fish. Milestone report, January 1995. Report 95-FRDCMS1. Report prepared for the Fisheries Research and Development Corporation.

Bakes, M. J. and Nichols, P. D. (1995) Lipid and fatty acid composition of oil produced from

Atlantic salmon waste. Report 95-Tassal1. Report prepared for Tassal.

Bakes, M. J. and Nichols, P. D. (1995) Lipid and fatty acid composition of oil from bream and carp. Report 95-Marchem1. Report prepared for Marchem.

Bakes, M. J. and Nichols, P. D. (1995) Proximate lipid and fatty acid composition of New Zealand shark liver oil. Report 95-C&FR1. Report prepared for the Crop and Food Research Institute.

Bakes, M. J. and Nichols, P. D. (1995) Proximate lipid and fatty acid composition of New Zealand shark liver oil. Report 95-C&FR2. Report prepared for the Crop and Food Research Institute.

Bakes, M. J. and Nichols, P. D. (1995) Proximate lipid and fatty acid composition of liver oil from spotted Wobbegong. Report 95-SC2. Report prepared for Scales.

Bakes, M. J. and Nichols, P. D. (1995) Lipid class composition of fish oil extracts. Report 95-SIA1. Report prepared for Solutions in Agriculture.

Bakes, M. J. and Nichols, P. D. (1995) Assay of lipid classes in hydrogenated shark liver oils supplied by CSIRO Division of Chemicals and Polymers. Report 95-C&P1. Report prepared for CSIRO Division of Chemicals and Polymers.

Nichols, P., Bakes, M. and Elliott, N. (1995) Marine oils from Australian fish. Risk assessment and consideration of the internal environment. Report 95-FRDC1. Report prepared for the Fisheries Research and Development Corporation.

Bakes, M. J. and Nichols, P. D. (1995) Supercritical fluid extraction as a tool for enriching marine oils: concentration of squalene and triacyglycerols. Report 95-CS3. Internal report.

Nichols, P. D. (1995) Lipid class composition of fractionated fish oil extracts. II. Report 95-SIA2. Report prepared for Solutions in Agriculture.

Nichols, P. D. (1995) Assay of hydrogenated shark liver oil fractions provided by CSIRO Chemicals and Polymers. Report 95-C&P4. Report prepared for CSIRO Division of Chemicals and Polymers.

Nichols, P. D. (1995) Lipid class composition of Scales shark liver oils. Report 95-SC3. Report prepared for Scales.

Nichols, P. D. and Bakes, M. J. (1995) Proximate lipid and fatty acid composition of New Zealand shark liver oil. Report 95-C&FR3. Report prepared for the Crop and Food Research Institute.

Nichols, P. and Bakes, M. (1995) Purification of omega-3 fish oils: Progress report. Report 95-CS5. Internal report.

Nichols, P., and Bakes, M. (1995) Purification of omega-3 fish oils: Progress report (Appendix). Report 95-CS5 (Appendix). Internal report.

Nichols, P., Bakes, M. and Strauss, C.(1995) Information on Patent 95CSD01. Report 95-CSD01. Internal report.

Nichols, P., and Bakes, M. (1995) Comparison of the lipid and fatty acid composition of "Chamotan" and Australian marine oils. Report 95-CS6. Internal report.

Bakes, M. J. and Nichols, P. D. (1995) Lipid and fatty acid composition of Ling liver oil. Report 95-BEKU1. Report prepared for BEKU Environmental Products.

Nichols, P. D. (1995) Examination of the lipid and fatty acid composition of the Clover "Milkarra" product. Report 95-CL1. Reported prepared for Clover Corporation Pty Ltd.

Nichols, P. D. (1995) Lipid class, fatty acid and fatty alcohol composition of BEKU New Zealand deodorized orange roughy oil. Report 95-BEKU2. Report prepared for BEKU Environmental Products.

Nichols, P. D. (1995) Lipid class composition of smoked salmon oil supplied by Scales. Report 95-SC4. Report prepared for Scales.

Bakes, M. J. and Nichols, P. D. (1996) Lipid and fatty acid composition of carp oil. Report 96-BEKU1. Report prepared for BEKU Environmental Products.

Bakes, M. J., Cablewski, T., Nichols, P. D., Simpson, G. W. and Strauss, C. R. (1996) CSIRO process for recovery of squalane from shark liver oil. Internal report.

Nichols, P. D. (1996) Squalene and Lipid Class Composition of Commercial Squalene Capsules. Report 96-BEKU2. Report prepared for BEKU Environmental Products.

Nichols, P. D. (1996) Lipid class and fatty acid composition of Clover tuna oil. Report 96-Clover1. Reported prepared for Clover Corporation Pty Ltd.

Nichols, P. D. (1996) Fatty acid composition of Wallace Bros. Heart Smart eggs. Report 96-WB1. Reported prepared for Wallace Bros.

Nichols, P. D. (1996) Lipid class and fatty acid composition of Clover Evening Primrose oil samples. Report 96-Clover2. Reported prepared for Clover Corporation Pty Ltd.

Nichols, P. D. (1996) Lipid class and fatty acid composition of BEKU trial and commercial emu oils. Report 96-BEKU3. Report prepared for BEKU Environmental Products.

Nichols, P. D. (1996) Oil content and lipid class composition of Clover Pacific tuna samples. Report 96-Clover3. Reported prepared for Clover Corporation Pty Ltd.

Nichols, P. D. (1996) Lipid class composition of BEKU trial emu and jack mackerel oils. Report 96-BEKU4. Report prepared for BEKU Environmental Products.

Nichols, P. and Bakes, M (1996) Lipid and fatty acid composition of oil produced from Atlantic Salmon waste. Report 96-GSO1. Report prepared for Groundswell Organics Tas

Pty Ltd.

Nichols, P. and Bakes, M. (1996) Lipid class composition of BEKU shark liver and orange roughy oils and squalene fractions. Report 96-BEKU7. Report prepared for BEKU Environmental Products.

Nichols, P.D. (1996) Proximate lipid and fatty acid composition of New Zealand Shark liver oil. Report 96-C&FR1. Report prepared for the Crop and Food Research Institute.

Nichols, D.S. and Nichols, P.D. (1996) Lipid class composition of BEKU squalene and New Zealand shark liver oil. Report 96-BEKU9. Report prepared for BEKU Environmental Products.

Nichols, D.S. and Nichols, P.D. (1996) Lipid class composition of BEKU purified wax ester oils. Report 96-BEKU10. Report prepared for BEKU Environmental Products.

Nichols, P. D. (1996) BEKU Environmental Products Ltd: Marine oils research and development. Report 96-BEKU5. Report prepared for BEKU Environmental Product

Nichols, P.D. and Quamby, T. (1996) Lipid class composition of BEKU wax ester fractions. Report 96-BEKU11. Report prepared for BEKU Environmental Products.

Nichols, P.D. (1996) Lipid class composition of BEKU DAGE (compared to solutions in Agriculture) and commercial wax ester fractions. Report 96-BEKU12. Report prepared for BEKU Environmental Products.

Nichols, P.D. (1996) Lipid class composition of BEKU shark liver oils. Report 96-BEKU13. Report prepared for BEKU Environmental Products.

Nichols, P. D. (1996) Oil content and lipid class composition of Clover US tuna samples. Report 96-Clover4. Report prepared for Clover Corporation Pty Ltd.

Mooney, B. and Nichols, P.D. (1996) Lipid class composition of BEKU shark liver oils. Report 96-BEKU14. Report prepared for BEKU Environmental Products.

Bakes, M. J., Nichols, P. D. and Strauss, C. R. (1996) Purification of omega-3 fish oils: Initial scale-up of process. Task 1 progress report. Report 96-Clover5. Report prepared for Clover Corporation Pty Ltd.

Nichols, P. D. (1996) Lipid class composition of Clover "Milkarra" capsules. Report 96-Clover6. Report prepared for Clover Corporation Pty Ltd.

Bakes, M. J., Nichols, P. D. and Strauss, C. R. (1996) Purification of omega-3 fish oils: Pilot plant trials. Task 2 Progress Report (1). Report 96-Clover6. Report prepared for Clover Corporation Pty Ltd.

Nichols, P. D and Mooney, B. (1996) Determination of lipid class, fatty acid and alkoxyglycerol composition of BEKU *TASMAN* Alkoxyglycerol. Report 96-BEKU18. Report prepared for BEKU Environmental Products.

Nichols, P. D. (1996) Report on overseas travel. United States. August 23 - September 12, 1996. Report 96-OS1.

Mooney, B. and Nichols, P. D. (1996) Composition of BEKU DAGE oil, Batch Da04126. Report 96-BEKU31. Report prepared for BEKU Environmental Products.

Nichols, P. D. (1996) Fatty acid composition of Clover "Milkarra" capsules. Report 96-Clover7. Report prepared for Clover Corporation Pty Ltd.

Mooney, B. and Nichols, P. D. (1996) Composition of oils under consideration by Clover. Report 96-Clover8. Report prepared for Clover Corporation Pty Ltd.

Mooney, B., Bakes, M., Strauss, C. and Nichols, P. D. (1997) Lipid class composition of oils from Clover scale-up trial, December 1996. Report 97-Clover1. Report prepared for Clover Corporation Pty Ltd.

Mooney, B., Bakes, M. and Nichols, P. D. (1997) Lipid class composition of Residue Samples from Clover, January, 1997. Report 97-Clover2. Report prepared for Clover Corporation Pty Ltd.

Mooney, B. and Nichols, P. D. (1997) Lipid class composition of BEKU DAGE oils. Report 97-BEKU1. Report prepared for BEKU Environmental Products.

Bakes, M., Strauss, C. and Nichols, P. D. (1997) Process for enrichment of fish oil-derived polyunsaturated fatty acids. Report 97-DC&P/MR1. Internal report.

Mooney, B., Bakes, M. and Nichols, P. D. (1997) Lipid class composition of oil samples from Clover, scale-up trials, January 27, 1997. Report 97-Clover3. Report prepared for Clover Corporation Pty Ltd.

Mooney, B., Bakes, M. and Nichols, P. D. (1997) Lipid class composition of oil samples from Clover, scale-up trials, January 29, 1997. Report 97-Clover4. Report prepared for Clover Corporation Pty Ltd.

Mooney, B. and Nichols, P. D. (1997) Lipid class composition of BEKU Pristane fraction. Report 97-BEKU7. Report prepared for BEKU Environmental Products.

Bakes, M., Mooney, B., Strauss, C. and Nichols, P. D. (1997) Degumming trials of Clover tuna oil. Interim Report 97-Clover5. Report prepared for Clover Corporation Pty Ltd.

Nichols, P. D. (1997) Lipid class composition of Clover American Samoan oil. Report 97-Clover6. Report prepared for Clover Corporation Pty Ltd.

Virtue, P., Mooney, B. and Nichols, P. (1997) Oil composition of Patagonian toothfish (*Dissostichus eleginoides*) flesh, oil and meal. Report prepared for Austral Fisheries.

Nichols, P. D. (1997) Fatty acid composition of Clover American Samoan oil. Report 97-Clover7. Report prepared for Clover Corporation Pty Ltd.

Nichols, P. D. (1997) Lipid class composition of Clover tuna oil (American Samoa, week 2) and enriched Evening Primrose oil. Report 97-Clover9. Report prepared for Clover Corporation Pty Ltd.

Nichols, P. D. (1997) Report on overseas travel. France and Italy. September 14 - October 1, 1997. Report 97-OS1.

Bakes, M. J., Nichols, P. D., Elliott, N. and Virtue, P. (1997) A preliminary study of the oils from selected fish Australian species: Trash and by-catch species. Report 97-DMR2. Internal report.

Conference and other presentations

Bakes, M. J., Nichols, P. D., Elliott, N. G. and C. R. Strauss (1995) Commercialization of waste marine oils in Australia. Australian Marine Science Association, July, Sydney, Abstracts.

Nichols, P. D. (1995) Progress in the development of marine oil products in Australia. The Royal Society of Tasmania. Invited lecture. Hobart, September.

Bakes M. J., Nichols P. D. and Strauss C. R. (1995) Developments with and commercialization of marine oils in Australia, International Chemical Congress of Pacific Basin Societies, Honolulu, Hawaii December.

Bakes, M. J., Elliott, N. J., Nichols, P. D. and Strauss, C. (1996) Value adding Australian marine oils. Australian Marine Science Association, July, Hobart, Abstracts.

Phleger, C. F. and Nichols, P. D. (1996) Lipids of Antarctic zooplankton and fishes from Elephant island. Australian Marine Science Association, July, Hobart, Abstracts.

Bakes, M. J., Elliott, N. J., Nichols, P. D. and Strauss, C. (1996) Value adding Australian marine oils. 2nd World Fisheries Congress, July, Brisbane, Abstracts.

By invitation, Michael Bakes also attended the symposium "Making the Most of the Catch" organized in conjunction with IFIQ which was held in Brisbane, July 1996.

Nichols, P. D. (1996) Developments with marine oil products and environmental biomarker research. Invited seminar, Department of Biology, San Diego State University, September, San Diego.

Nichols, P. D. (1996) Lipid Biomarkers. Invited seminar, Centre for Environmental Biotechnology, University of Tennessee, September, Knoxville.

Bakes, M. J., Elliott, N. G., Nichols, P. D. and Strauss, C. (1996) Value adding Australian marine oils. Australian Organic Geochemistry Conference, October, Perth, Abstracts.

Phleger, C. F., Nichols, P. D. and Virtue, P. (1996) Southern Ocean pteropods: a potential buoyancy role for diacylglycerol ether. Soc. Integrative and Comparative Biology. Ann. Meeting, December, Albuquerque, Abstracts.

Dundas-Smith, P. and Nichols, P. (1997) Fisheries Research and Development Corporation. Inaugural Workshop, Australasian Section of the American Oil Chemists Society. February, Canberra.

Nichols, P., Bakes, M., Mooney, B. Elliott, N. and Strauss, C. (1997) Progress with marine oil products in Australia. Inaugural Workshop, Australasian Section of the American Oil Chemists Society. February, Canberra.

Nichols, P. D., Nichols, D. S., Lewis, T., Brown, J., Bowman, J. P. and McMeekin, T. A. (1997) Novel bacteria as alternate sources of polyunsaturated fatty acids for use in aquaculture and other industries. Marine Microorganisms for Industry conference, September, Brest, Abstracts.

Bakes, M., Nichols, D. S., Nichols, P. D., Strauss, C., Bode, O. and Drummond, G. (1997) Developments with omega-3 polyunsaturated fatty acid oils in Australia. Fourth International Marine Biotechnology Conference, September, Sorrento, Italy, Abstracts.

Nichols, P., Bakes, M., Mooney, B. Elliott, N. and Strauss, C. (1997) Developments with marine oil products in Australia. Pacific Oils 2000, Auckland, New Zealand, Abstracts.

Virtue, P. Mooney, B., Elliott, N., Bakes, M., Nichols, P., Bode, O., Anderson, R. and Drummond, G. (1997) Pacific Oils 2000, Auckland, New Zealand, Abstracts.

Bakes, M. J., Elliott, N. G., Nichols, P. D. and Strauss, C. (1997) Value-adding Australian marine oils. Pacific Oils 2000, Auckland, New Zealand, Abstracts.

Patents

Nichols, P. D., Nichols, D. S., Simpson, G. and Strauss, C. "Separation Process" Provisional Patent for application to shark liver oils lodged November 1993. Maintained as confidential know-how since November 1994.

Nichols, P. D., Bakes, M. J. and Strauss, C. "Purification Process". Provisional patent lodged January 1995, refiled January 1996 and January 1997.

APPENDIX B

Report 95-FRDC1

Confidential

REPORT, MAY 1995

FISHERIES RESEARCH AND DEVELOPMENT CORPORATION

PROJECT FRDC 94/115

MARINE OILS FROM AUSTRALIAN FISH: CHARACTERIZATION AND VALUE ADDED PRODUCTS

Risk Assessment and Consideration of the International Environment

Peter Nichols[1]
Michael Bakes[1]
Nick Elliott[2]

[1]CSIRO Division of Oceanography
[2]CSIRO Division of Fisheries

GPO Box 1538, Hobart, Tasmania 7001

Risk Assessment and Consideration of the International Environment

During year 1 of FRDC project 94/115, research was performed as stated in the objectives of the original proposal. The project objectives remain the same (see ongoing application) and good progress has been achieved as outlined in the first project milestone report (Division of Oceanography Report 95-FRDCM1; attachment). For consideration of the ongoing application of project 94/115, the Board needed inclusion of a rigorous risk assessment and consideration of the commercially competitive international environment in which the local producer must operate.

The further input outlined below includes, as requested (appendix):
1. technical assessment re achievements of objectives,
2. analysis of environmental factors impacting on the marine oils industry,
3. assessment of issues on a more specific/micro level.

1. Technical assessment re achievements of objectives

The objectives are centered in two areas: (i) the ongoing characterization of Australian marine oil resources and provision of these results to the scientific community and industry, and (ii) Examination and optimization of methods for further refining squalene-containing and omega-3 containing marine oils, thereby increasing product value. The status of research conducted to date is also briefly summarized in non-technical terms in the taped interview enclosed with this report. Positive feedback for future R&D has resulted from a range of similar interviews around Australia.

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. The three types of oils available from Australian species are:
1. the wax ester oils,
2. squalene-rich (and diacylglycerol ethers: DAGE) oils,
3. triacylglycerol (TAG) oils, containing omega three polyunsaturated fatty acids (PUFA).

The funding provided by FRDC 94/115 allowed this expertise, in many cases otherwise not available, to be used by the developing Australian Marine Oil industry.

The research conducted to date has assisted a number of companies that have established facilities for the production of oil products from the south-east Australian fisheries. Numerous requests have been received from industry around the country regarding the characterization of Australian oils, and in all cases the highest quality analytical data has been provided (Report 95FRDCM1). The need for ongoing data on the oil composition of new species is considered essential. In several instances in dealing with the industry, it has been necessary to correct anomalies that have arisen over the composition of various marine oil raw materials and products (e.g., raw material composition, incorrect labeling). In the commercially competitive international environment, the provision of accurate technical data and advice is essential and this component of the research would be an ongoing part of project 94/115.

The second part of the project is related to the examination and optimization of methods for further refining marine oils. Progress has included:

(i) As part of ongoing research emanating from project 91/77, the squalene process developed by the Divisions of Oceanography and Chemicals and Polymers was licensed to industry. An agreement was completed in July 1994 and refinement of the costing of the process was undertaken by a sub-licensee; production and export of squalene has been commenced. At this stage, based on the export of product by industry using the CSIRO process and by another company to which advice has also been provided, the squalene processes in use by Australian industry appear to be technically sound.

(ii) The development of a laboratory process to purify omega-3 PUFA fish oils (see below for further details on the transfer of this technology). The process has been developed jointly by the Divisions of Oceanography and Chemicals and Polymers. It is envisaged that the process could be used to add-value to crude oils currently produced in Australia (e.g. tuna oil). A provisional patent was lodged in January 1995. Further refinement of this process is required during years 2 and 3.

(iii) A process to produce and separate squalane (produced after hydrogenation of squalene) has been developed by the Division of Chemicals and Polymers with the assistance of project 94/115. Further refinement at a laboratory scale of the newly developed squalane process will be required as part of years 2 and 3 of the project. In addition to optimizing squalane recovery and purity, the production and isolation of saturated DAGE/TAG also needs to be optimized. The potential use of the saturated DAGE/TAG by-product also would need to be investigated (e.g. in mariculture feeds). Scaling-up of the squalane process will need to be examined, both at a larger laboratory scale and when progress is sufficient, in the pilot facility at the Division of Chemicals and Polymers.

(iv) We have developed a laboratory procedure to isolate DAGE from shark liver oil. This process will be of relevance to the second component of the squalane research.

(v) The trialling of supercritical fluid extraction (SFE) equipment for oil separation has been completed. SFE is a relatively new form of technology that shows potential for the purification and possible enrichment of marine oil products. The trial results look promising and further research would be pursued in years 2 and 3.

Future process oriented research will focus initially on the squalane and omega-3 procedures. Good technical progress has been demonstrated with the squalene process, and, with other squalene developments within the Australian industry. The likelihood of further technically sound developments is considered good, as a flow-on from future research provided through project 94/115.

2. Analysis of environmental factors impacting on the marine oils industry

The key factor for the marine oils industry is the fisheries resource. The resource is dependent on sustainable catches and the need for better utilization of the waste and by-products. Consultation with various fisheries bodies (e.g. AFMA, NT DPIF, CSIRO Division of Fisheries) during preparation of this report revealed or confirmed several features:

(i) The deep-sea shark resource (by-catch of other fisheries, source of squalene, squalane, DAGE oils) has not been surveyed and no stock assessment information is available. However, based on consultation with industry and management bodies, the likely catch rate required to meet the current and predicted needs of the Australian Marine Oil industry is

believed to be sustainable. This scenario will need to be monitored.

(ii) The southern blue fin and yellow fin tuna fisheries (waste from processing, source of omega-3 oils) are also considered sustainable by the various bodies. Currently Australia does not produce any purified omega-3 products. Raw material is already available; e.g. the Eden plant produces between 100-200 tonnes p.a.. At Triabunna production of between 1000-2500 tonnes p.a. of oil (from jack mackerel) has been achieved. The ongoing sustainability of these resources is vital for any of the planned development opportunities with the Australian omega-3 oils.

(iii) The orange roughy fishery (waste from processing, source of wax ester oils) quota has been greatly reduced in recent years. At whatever level is sustainable for this fishery, use of the waste oil will maximize the value of the catch. As a strategy to counter the decrease in the raw oil resource, the oil from imported roughy (for value-adding in Australia) has been surveyed for wax esters as have other species (e.g. oreos) and the results have been communicated to industry and in scientific forums. The composition of oils from other deep-sea species is planned to be surveyed in years 2 and 3.

The oil resource is considered adequate for Australian industry to meet predicted local and export demands. Developments with the characterization of new oil resources and with the ensuing appropriate purification processes will be factors industry will rely on.

3. *Assessment of issues on a more specific/micro level.*

Input from industry has been provided to also cover this point (Appendix).

Strengths for the Australian Marine Oil industry include:

- the sustainability of the raw material (in comparison, several competing countries show indications that raw resources are in decline),
- the clean and green resource (c.f. competitors),
- the demonstrated dedication of the Australian industry, including the building of the raw material supply network,
- closeness to major markets (Asia),
- a preference by certain buyers to deal with Australian rather than traditional suppliers,
- the uniqueness (and underutilization) of the composition of several of Australia's marine oil resources,
- the development of political connections by sectors of the Marine Oil industry
- emergence and growth of the Australian Marine Oil industry,
- a good technical knowledge of the composition of local marine oils (and in some instances of competitors oils) has been obtained through the assistance of FRDC funding ,
- the now on-line development of appropriate technologies, again including through the assistance of FRDC funding .

Weaknesses include:

- the monopoly of certain areas of the marine oil market by overseas competitors,
- the lack of financial resources for many of the Australian Marine Oil Small and Medium Enterprises, and therefore the ability to conduct R&D (to our knowledge, most of the companies recently entering the market have yet to trade in the black)
- in the case of certain products, the non-compliance of the raw material with specifications,

- the difficulty for the emerging Australian industry to break into the market,
- the long lead time to enter the market,
- possible inexperience of Australian companies in dealing with buyers of vastly different cultures. These last three weaknesses are clearly related.

Opportunities based on many of the strengths have now been demonstrated and the Australian industry is considered well placed to meet the challenge of breaking into the market place for several marine oil products.

- the opportunity for developing new products exists in some instances, based on the uniqueness of the raw resource, including the clean and green image.
- already there is evidence that initial shipments of several Australian products (e.g. squalene) have been well received and have the potential to capture market from competitors.
- the ability of Australian industry and researchers to develop appropriate technologies and products for the future market,
- together these and other features will provide the Australian fishing industry with the opportunity to improve its image through better utilization of existing, and, other waste resources.
- the image of FRDC would continue to benefit from an association with R&D directed to minimizing waste of the by-catch and from processing.
- the involvement of an independent research organization (CSIRO) in R&D would be beneficial to a variety of organizations.

The opportunities and the raw resources now exist for the production on a per annum basis of initially 10-20 tonnes of squalene; this could expand to 100-200 tonnes over several years. The world market is estimated at between 1000-2000 tonnes p.a. At market value, the initial Australian squalene production is worth around $0.5M, this value will increase by up to a factor of 10 or more as production increases.

World production and estimates of the potential national and export market are higher for the squalane and omega-3 TAG oils. Presently, further R&D is required to bring about Australian production. For the omega-3 oils, using the yield information presented above for the raw resource and an initial estimate of 20-30 tonnes production, the return would be $1.3M. This value would again be expected to increase as production increases.

The main *threats* are considered to be:

- the continued dominance, and therefore possible control, of the market by competitors,
- the reluctance of some areas of the fishing industry to support this new industry through the continued dumping of by-catch and process waste,
- inappropriate government legislation (e.g. Therapeutic Goods Act regulations with respect to squalene; banning of harvesting/landing of certain dogfish species due to historical factors, for example, deep sea dogfish in Victoria based on heavy metal information),
- fluctuations in the currency market.

In summary, the Australian Marine Oil industry has made rapid progress. The opportunities are available now for consolidation of developments to date and the commencement of new initiatives. The industry is clearly in need of further R&D. The ongoing characterization and process oriented research planned for years 2 and 3 of Project 94/115

will complement and strengthen industry initiatives and will allow the Marine Oil and kindred industries to maximize the return for the Australian fishing industry.

Acknowledgment

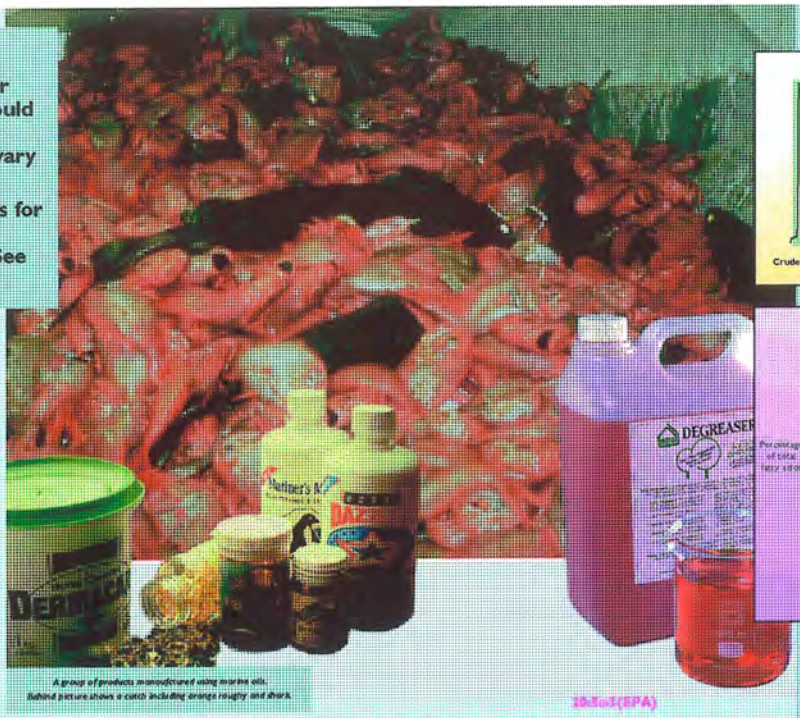
We gratefully acknowledge the support and interest in the research shown by the Australian Marine Oils industry; the input to the project by CSIRO Division of Chemicals and Polymers colleagues (Chris Strauss, Teresa Cabelski, Greg Simpson); the financial support of the FRDC without which the R&D would not have been possible.

VALUE-ADDING AUSTRALIAN MARINE OILS

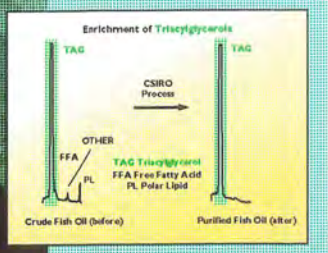
BACKGROUND

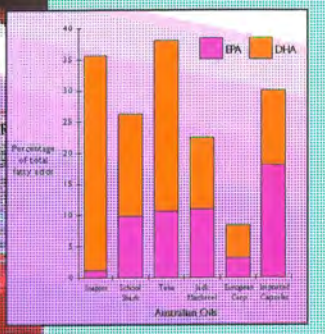
Amazingly, around 100 000 tonnes per annum of the Australian fish catch would normally go to waste. However, fish contain a diverse array of oils, which vary markedly between species.

We are isolating valuable components for cosmetics, dietary supplements, pharmaceuticals and industrial use. See the examples below.



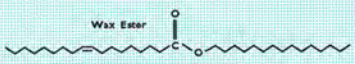
A group of products manufactured using marine oils. Behind pictured are a cod, including orange roughy and shark.





WAX ESTER OILS

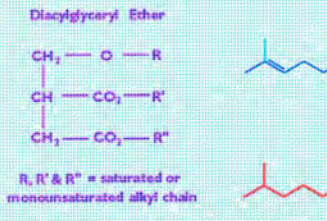
- similar to Jojoba and sperm whale oil, only better !
- new market opportunities:
  - cosmetics (shampoos & moisturisers)
  - pharmaceuticals (treatment of acne, psoriasis, eczema and sunburn)
  - degreasers, hand cleaners and lubricants

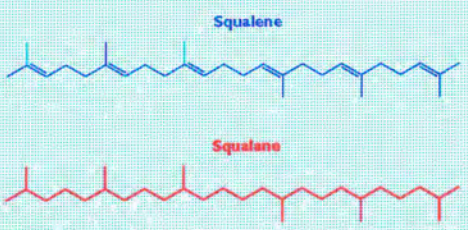


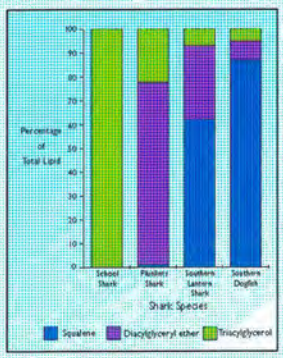
HYDROCARBON OILS

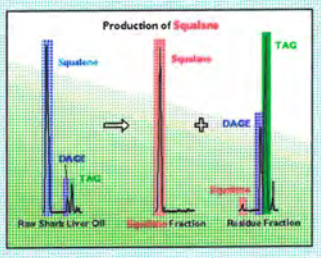
Shark liver oil is a source of squalene, triacylglycerols and diacylglyceryl ethers

- **Squalene now produced in Australia for cosmetics, lubricants, and in health and pharmaceutical products.**
- **Squalene is converted to squalane, used as a lubricant and in cosmetics.**









OMEGA-3 OILS

Omega-3 polyunsaturated fatty acids (ω3-PUFA), such as eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3) are found in oil from most fish species.

- **They are believed to reduce the risk of coronary heart disease and other disorders (eg. dyslexia, atherosclerosis, childhood asthma). They are also beneficial for brain and retina development and function.**
- **Available in triacylglycerol form as imported capsules and bottled oil in supermarkets, health food stores and chemists.**

SUMMARY

Current research and development

- **nutritional survey of Australian commercial seafoods**
- **characterisation of marine oils (includes fish, algae, bacteria)**
- **process development**

How may we help you with a new product line or improve the return on your catch ?

ACKNOWLEDGEMENTS

We work closely with Australian industry and particularly acknowledge support from the Fisheries Research and Development Corporation, BEKU Environmental Products, Squalus and Clover Corporation.

This poster was designed by Michael Bessell

OCEANOGRAPHY

MICHAEL J. BAKES[1,2]
NICHOLAS G. ELLIOTT[3]
PETER D. NICHOLS[1]
CHRISTOPHER R. STRAUSS[2]

CSIRO CHEMICALS AND POLYMERS[2]
CLAYTON, VICTORIA

DIVISION OF FISHERIES

CSIRO FISHERIES[3]
HOBART, TASMANIA

CSIRO DIVISION OF OCEANOGRAPHY[1]
GPO BOX 1538
HOBART TAS 7001
AUSTRALIA

Oil Composition of Australian Seafoods: Tuna Oils Rich in DHA

Background

The content and composition of the oil from Australian fish, shellfish and crustaceans were examined to:

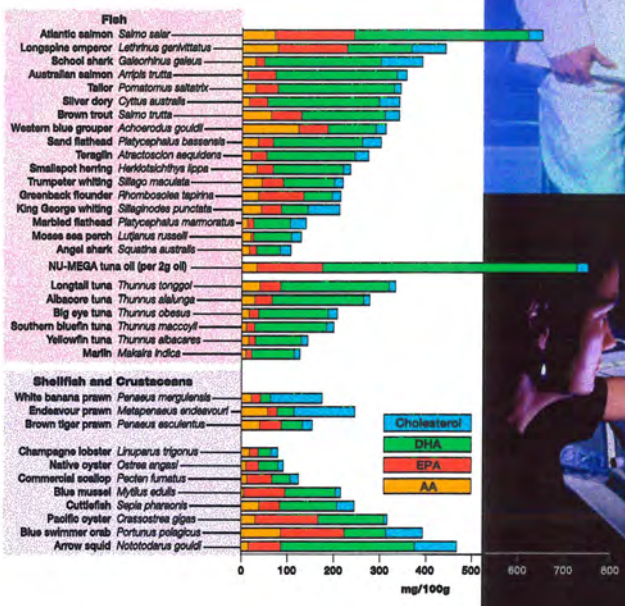
- Determine the nutritional value of these species.
- Investigate whether oil levels differ with region, species groups and other factors.
- Transfer know-how to industry to better exploit the total catch, including waste products from tuna.

Oils and nutrition

EPA (eicosapentaenoic acid, 20:5ω3) and DHA (docosahexaenoic acid, 22:6ω3) are omega-3 polyunsaturated fatty acids (PUFA). They are essential in the human diet and help reduce the risk of coronary heart disease, stroke, asthma and other disorders, including possible activity against cancer, dyslexia and dementia. Omega-3 fatty acids are also beneficial for brain and retina function and development. Nutritional studies indicate DHA is more active than EPA for certain disorders.

AA (arachidonic acid, 20:4ω6) is an omega-6 fatty acid which is a precursor of prostaglandins and other important compounds.

Cholesterol is a structural component of many tissues. It is synthesised by humans, but in excess is a precursor of coronary heart disease and other disorders.



Research findings

- **Most Australian fish have high levels of the omega-3 PUFA relative to other food groups and low levels of cholesterol (representative data is shown).**
- **Prawns have lower levels of omega-3 PUFA and higher levels of cholesterol compared to fish.**
- **Australian fish generally have higher relative levels of DHA than fish from northern hemisphere waters including oil products from these fishes.**
- **Tuna are a particularly good source of DHA-rich oil.**

Industry outcomes

NU-MEGA LIPIDS commenced manufacture of Australian-made, value-added tuna oil products in 1997. The tuna oil products contain 25% DHA, a DHA to EPA ratio of >3, and a lower proportion of cholesterol than is found in flesh from tuna and other seafood.

Patti Virtue Ben Mooney Nick Elliott
Peter Nichols Chris Strauss Michael Bakes
Guy Drummond Rob Anderson Ortwin Bode

Acknowledgments
Research supported by Fisheries Research and Development Corporation. Poster design by Michael Bessell (1997)

CSIRO MARINE RESEARCH

FISHERIES RESEARCH & DEVELOPMENT CORPORATION

NU-MEGA LIPIDS

Polyunsaturated Fatty Acids from Sea Ice Microbiota

Lipid deposits (yellow) within sea ice diatoms visualised by Nile Red stain.

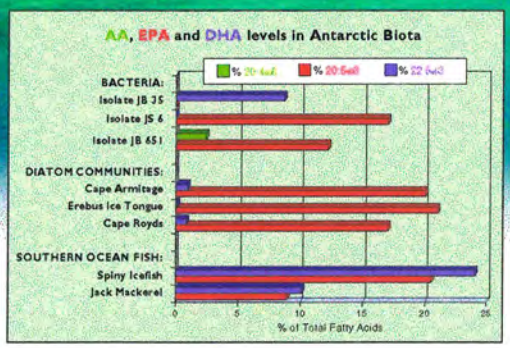
Scanning electron micrograph of sea ice bacteria.

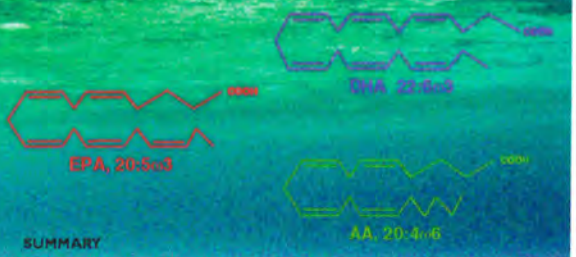
Every year, vast areas of sea ice form and melt around Antarctica. Ice coverage extends from 3 million km^2 in February, to 20 million km^2 in September/October. Sea ice supports the growth of microalgae (mostly diatoms) together with bacteria.

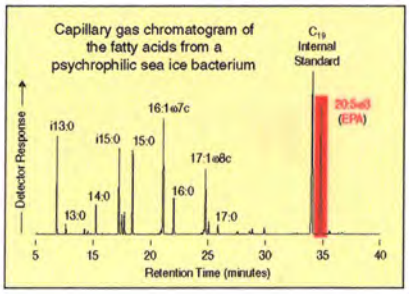
An important role of the microbes, for higher trophic levels, is the biosynthesis of polyunsaturated fatty acids (PUFA) such as eicosapentaenoic acid [20:5ω3 (EPA)] and docosahexaenoic acid [22:6ω3 (DHA)].

Sea ice microbiota are important sources of these essential components for marine organisms.

This study investigates fatty acid composition of sea ice diatom communities during a summer bloom in McMurdo Sound, and examines PUFA production by psychrophilic sea ice bacteria isolated from Prydz Bay. PUFA production has been observed for only a limited number of bacteria and has not been reported previously from Antarctic bacteria.







SUMMARY

Diatom Communities:

- Diatom communities - high levels of PUFA (40–50% of fatty acids) with an average level of EPA at 19%; similar to pure cultures of Antarctic and temperate diatoms.
- The relative level of EPA varied with time, suggesting the level of EPA may respond to environmental factors.

Bacterial PUFA:

- Of over 110 psychrophilic strains, 23 produced EPA in the range of trace to 20% of fatty acids. Another 21 produced PUFA such as 18:2ω6, 18:3ω3 and 20:4ω3. Five strains produced DHA, the first strains to do so not isolated from the deep-sea. One strain produced 20:4ω6 (arachidonic acid, AA) and EPA.
- The proportion of PUFA-producers is an order of magnitude higher than in temperate environments; the constant low temperature of the Antarctic environment has selected strains maintaining membrane fluidity by producing PUFA.

Bacterial Hydrocarbons:

- Novel n-C$_{31:9}$ polyene in PUFA-producing strains, the first report of this hydrocarbon. It may be derived from EPA and represent membrane adaptation to low temperature.

Biotechnological Implications:

- Utilising bacteria as aquaculture feeds had not been pursued previously due to their perceived lack of PUFA. However, sea-ice bacteria may produce high levels of PUFA, allowing them to be possibly utilized in aquaculture feeds.
- Bacterial PUFA production is a "guaranteed" resource, in comparison to variable wild fisheries. Culturing bacteria is also cheaper and easier than algae for PUFA production.
- Industrial usage of purified PUFA (such EPA and DHA) as health products, food additives or pharmaceuticals is increasing. The majority of PUFA-producing bacteria accumulate one type of ω3 PUFA; usually EPA or DHA. This feature removes the cost of purifying individual ω3 PUFAs from the complex mixtures present in fish oils or algae.
- Successful feeding trials with rotifers have been completed. Rotifers were enriched in ω3 PUFA when fed an Antarctic bacterium.

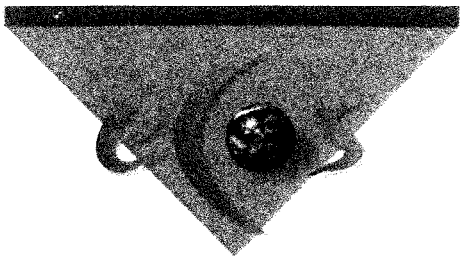
ANTARCTIC CRC

MARINE RESEARCH

David S Nichols[1], Janelle Brown[1], John Bowman[1], Jennifer H Skerratt[1,2], Peter D Nichols[1,2], Tom A Mcmeekin[1] and Cornelius W Sullivan[3]

[1] Antarctic CRC & Dept. Agricultural Science, University of Tasmania, Hobart, Tas, Australia;
[2] CSIRO Division of Marine Research, Hobart, Tas, Australia;
[3] Hancock Institute for Marine Studies, Dept. Biological Sciences, Univ. Southern California, Los Angeles, CA, USA.

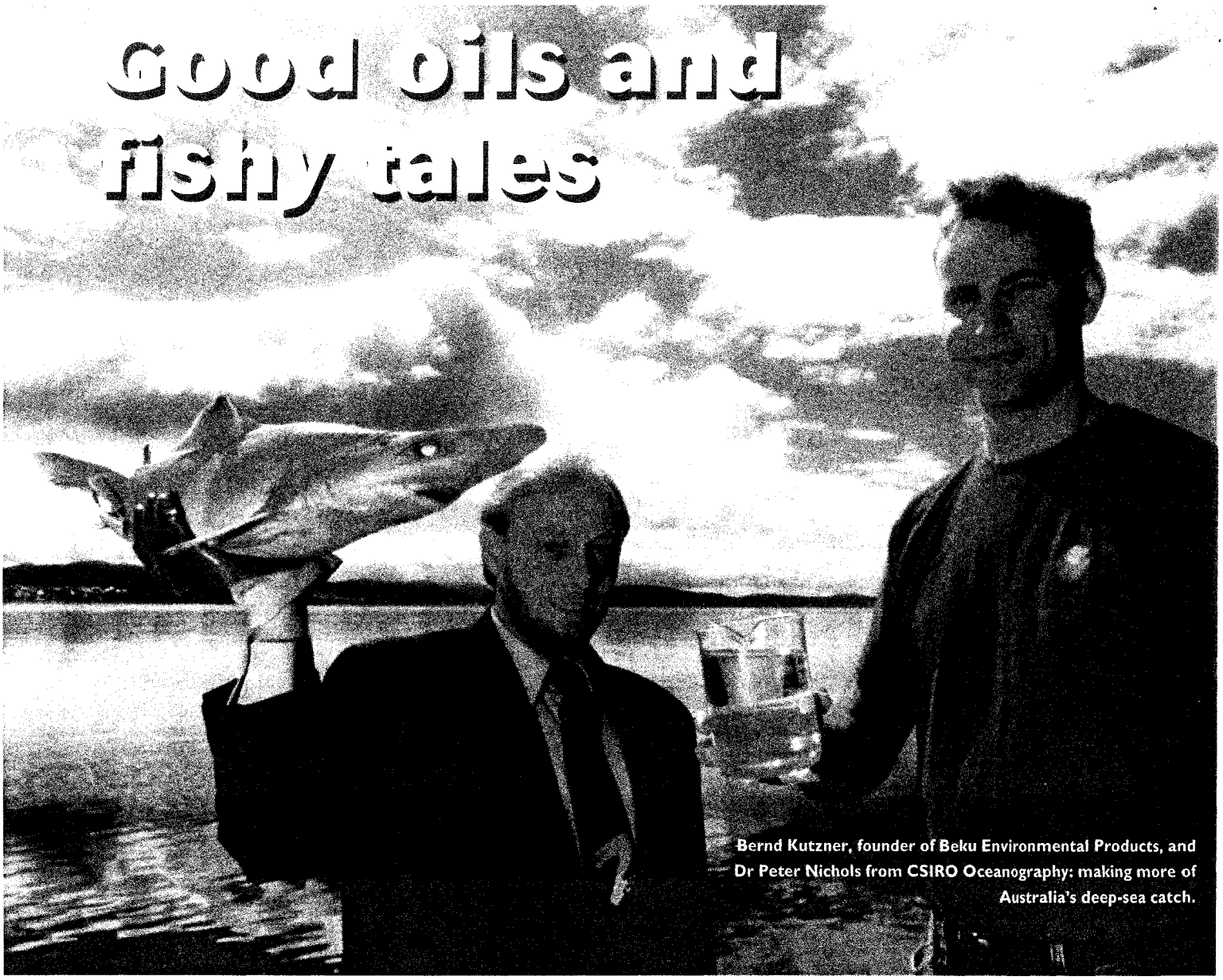
Acknowledgement
This work was supported by NSF DPP funding to A.C. Palmisano and CWS, APRA and ARC funding to DSN and JB, ASAC support to DSN, PDN, TAMcM and H Burton, FRDC funding to PDN. Our Antarctic CRC colleagues are thanked for help during the project. We also thank Michael Bestell who designed this poster.



This article is reprinted from
***Ecos,** CSIRO's*
quarterly Science and
Environment magazine.

For more information and
subscription details contact
CSIRO Publishing
PO Box 1139
Collingwood 3066
Victoria Australia
Phone: 03 9662 7500
Fax: 03 9662 7555
email: sales@publish.csiro.au

Good oils and fishy tales



Bernd Kutzner, founder of Beku Environmental Products, and Dr Peter Nichols from CSIRO Oceanography: making more of Australia's deep-sea catch.

Scientists and entrepreneurs are working together to boost the value of south-east Australia's deep-sea fisheries. Gill Shannon reports.

If the wheel were to be reinvented, would it happen at a small factory in the suburbs of Hobart?

Spend 10 minutes talking to Dan Cudmore, marketing director of Tasmanian company Beku Environmental Products Ltd, and you might believe it possible.

Cudmore will tell you all about the products that have in four years propelled the company out of a home garage and onto the export market, so successfully that in 1995 Beku won best new exporter in the Tasmanian division of the Australian Export Awards.

Beku is one of a number of enterprising Australian companies with their sights set on markets in China, Indonesia, Taiwan, Japan and Vietnam, as well as Europe and the United States in the long term. What is the product they are banking on?

Take Cudmore at his word, and you'd think they were marketing in various forms the elixir of life. Actually, their goods are based on oils salvaged from the least-favoured parts of dead deep-sea fish.

Beku's product range falls into three categories which, curiously, roughly align with dictionary definitions for elixir. They are:

- an industrial degreaser and a metal cutting, tapping and drilling fluid (a liquor once supposed to have the power of transmuting metals);
- an emollient that can be used as a base for cosmetics such as skin creams and lipsticks (a powder for wounds; a panacea); and
- a general health tonic (a liquor once supposed to have the power of indefinitely prolonging life).

Beku's first product, the degreaser, was launched in 1991 following two years of research and development led by CSIRO's Division of Oceanography and company founder, Bernd Kutzner. The resulting processing technique has been patented worldwide.

The degreaser, a hand cleanser, and a soluble cutting oil due to debut this year, are based on wax esters derived from oreo dory and orange roughy. Cudmore says the

products contain properties much like the whale oils which were put to similar use until Australia's whaling industry was halted some 40 years ago. Mineral and vegetable oils have been used since, but involve problems relating to biodegradation, toxicity and bacterial build-up.

'Our range represents a world-first alternative to volatile, petrochemical-based hand cleansers, degreasers and cutting oils,' Cudmore says. 'We're reinventing the wheel with these marine-oil products.'

Enriching the catch

Determining the chemical composition of oils contained in various fish wastes is crucial to the product-development process. This work is done by scientists at CSIRO's Division of Oceanography in Hobart as part of its Marine Products Project led by Dr Peter Nichols. The oceanography scientists work closely with Dr Chris Strauss and Michael Bakes at the Division of Chemicals and Polymers in Melbourne, and Dr Nick Elliott from CSIRO Fisheries in Hobart.

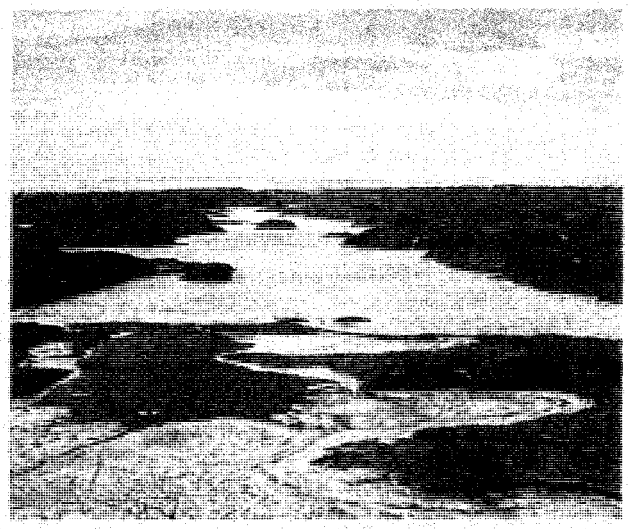
CSIRO Oceanography's research and development on marine oils, begun in the 1980s, involves scientists working with industry to seek profitable uses for the by-catch and by-products of Australia's south-eastern fisheries (such as orange roughy, jack mackerel, blue grenadier and deep-sea shark). Companies to benefit from this collaboration include Beku, Clover Corporation Pty Ltd, Squalus Pty Ltd and Scales Fish and Bait Sales.

Manager of Scales, Richard Saul, says the company buys once-discarded waste products which are then converted to raw fish oil. He says the material sourced by Scales is drawn from lower value by-catch species which were previously of little or no worth.

'Research into uses for the oils available from such species and other discarded material is assisting industry to improve the ulilization of these Australian marine resources,' Saul says. 'CSIRO's Marine Oil Program is helping us to establish an industry with benefits not only for the fishing sector but the broader community.'

One of the first products studied by the research group was orange roughy waste. Of the orange roughy catch, approximately 30% is processed into fillets, the remainder being discarded as waste. Traditional low-value uses of fish waste include pet food, fertiliser, bait, fish meal and unrefined oils.

Characterising oils from fish waste. In the laboratory the oil is first extracted with a solvent which in turn is removed, leaving the oil. Bacteria and microalgae (which produce the oils incorporated by the fish) are also grown in cultures to obtain suitable amounts for analysis. The oil is then analysed to the molecular level using various analytical and instrumental techniques. In this picture, Dr Patti Virtue examines oil composition.



Oils from icy seas

Scientists at the Division of Oceanography, in addition to their shark and orange roughy oil research, are studying the potential of other marine organisms as sources of omega-3 oil. These are Antarctic krill, algae and free-floating bacteria.

The division is studying these ocean dwellers in association with the Antarctic Cooperative Research Centre, which in 1986 established the Australian Collection of Antarctic Micro-organisms. The collection provides a foundation for the potential commercial exploitation of microbes from Australia's cold, Antarctic waters.

In studies complementing the division's research into marine oils from fin fish, bacteria have been isolated that produce polyunsaturated omega-3 fatty acids. This work is being conducted as part of the PhD program of David Nichols at the University of Tasmania.

Nichols says many organisms contain high levels of the essential polyunsaturated fatty acids eicosapentaenoic acid (EPA, 20:5(3) and docosahexaenoic acid (DHA, 22:6(3) at low temperatures. New strains of bacteria isolated from Antarctic waters have therefore been examined.

Early results indicate that the proportion of Antarctic strains that produce EPA is considerably higher than found for temperate marine bacteria. Similarly, a number of strains that produce DHA have also been isolated.

Future research in this area will focus on lifeforms from an even colder environment: psychrophilic bacteria isolated from the Antarctic sea-ice. In time, chemicals such as essential fatty acids made by microalgae and bacteria could be obtained and purified by similar technology to that developed for the production of value-added products from fish oils.

The Antarctic bacteria have been used successfully in aquaculture feeding experiments with rotifers. Rotifers are normally enriched with EPA and DHA with an algal diet prior to feeding in aquaculture operations. The bacteria may be an alternative to algae as a feed for rotifers in future.

Studies of orange roughy waste revealed that some 18% of the fish is oil, 80% of which exists in waste products: the swim bladder, frame and skin. They also found that orange roughy oil, unlike that of most other commercial fish, is composed almost exclusively of wax esters, one of three classes of lipids presently sought in marine oils. The others are squalene-rich oils (and diacylglycerol ethers) from shark livers and triacylglycerol oils containing omega-3 polyunsaturated fatty acids.

Beku forsees a great potential for squalene, which is a popular health tonic in Asia. Cudmore says 1500-1800 tonnes of squalene is used worldwide every year. The company has tapped a tiny portion of the market with its export of 40-70 tonnes annually for encapsulation. In addition, it uses only 1-2% of the available resource.

The squalene exported by Beku and other companies is extracted and refined from the livers of deep-sea sharks. These sharks, as well as being fished in their own right, are an under-utilised by-catch of orange roughy and other deep-water fisheries. Their livers are large (about 20% of the total shark's weight), and can contain considerable quantities of oil. In the past, Australian shark liver oil was sold unprocessed to Japan for value-adding.

In a further process, called hydrogeneration, squalene can be converted to squalane, which has a variety of uses in the pharmaceutical and cosmetic industries, particularly as a lubricant, tablet coating and cosmetic-base oil. The divisions of Oceanography and Chemicals and Polymers have developed a process to produce squalane from shark liver oil and will be seeking industry partners in 1996.

Omega-3 oils are also of interest to Beku and the company is working closely with CSIRO on their chemical characterisation. Research into the health benefits of omega-3 oils is being conducted by CSIRO's Division of Human Nutrition in Adelaide.

Omega-3 fatty acids are polyunsaturated fats most readily found in fish and fish oils. Small amounts, of a different kind, are also found in linseed oil, soya bean and canola oil. The acids feed into various pathways in the human body with widespread effects. They have been shown to:

- reduce blood triglyceride levels (trigylycerides are a type of blood fat that plays some role in the development of heart disease);

Fatty acids essential for fish

Rising demand for marine oils to include in feeds for the aquaculture industry is the driving force behind CSIRO's Aquaculture Nutrition and Biotechnology Project based at the Division of Oceanography.

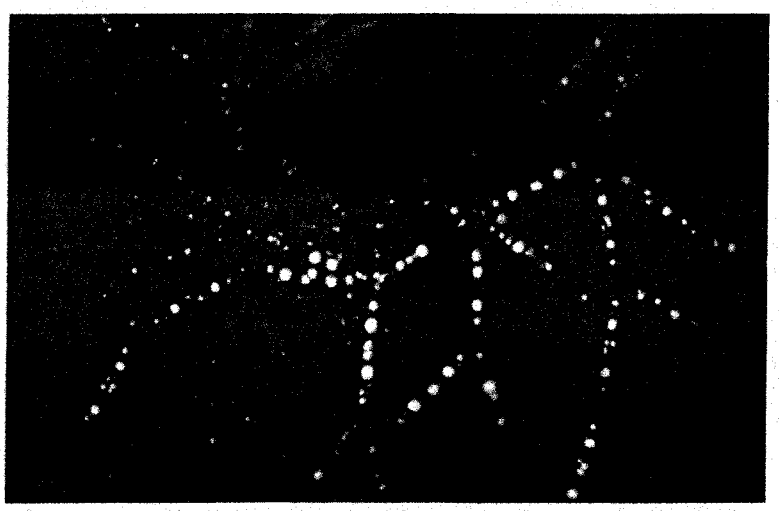
Project leader, Dr John Volkman, says marine oils containing two long-chain polyunsaturated fatty acids (eicosapentaenoic acid and docosahexaenoic acid) which are essential dietary components for most species reared by the aquaculture industry, both in Australia and overseas.

One of the main live feeds used in the aquaculture industry is microalgae, the staple food for many shellfish, and for prawns, crabs and crayfish during their early life stages. Microalgae are also an important food source for zooplankton such as copepods and rotifers (a food source for juvenile fish).

Graeme Dunstan and Stephanie Barrett have been studying the suitability of various microalgae as feeds for commercially important species. Dunstan has analysed selected strains of nutritious microalgae provided by colleagues from the CSIRO Division of Fisheries, and has identified several which contain high levels of polyunsaturated fatty acids. These are now used in Australian aquaculture hatcheries.

Dunstan is also investigating the dietary importance of lipids, particularly with respect to the early stages of the life cycles of a variety of marine animals such as abalone, where the greatest cost to industry is feed. He has shown that the growth rates of abalone are directly affected by the amount and type of polyunsaturated fatty acids in the diet.

Another potential feed source rich is polyunsaturated fatty acids is bull kelp, Durvillaea potatorum, a type of algae which is storm-cast in vast quantities on the beaches of Tasmania. Bull kelp is exported to Scotland to produce alginate, but could well be utilised as a feed for the aquaculture industry or in the pharmaceutical industry. Its lipid content is being studied by Dr Patti Virtue.



An alga from the Antarctic sea ice. The Nile red stain highlights lipid (yellow) stores produced under certain growth conditions against the background of chloroplasts (red). Methods for extracting and purifying the essential fatty acids contained in microalgae and bacteria are being developed.

In other aquaculture-directed research, the krill species Nyctiphanes australis, the most important food for a variety of abundant fishes in Tasmanian waters, is being examined. Krill are used extensively in feed for farmed salmon and other fishes in Japan and Canada. Virtue believes Nyctiphanes australis is ideal for farmed salmon as it has a near optimum range of essential fatty acids.

Much of the research described in this article has been co-funded by CSIRO and the Fisheries Research and Development Corporation and has been assisted by collaboration of colleagues in the aquaculture industry.

Marine oils: sources, markets and uses

lipids	wax esters	squalene-rich oils and diacylglycerol ethers (DAGE)	triacyglycerol oils (omega-3 polyunsaturated fatty acids:eicosapentaenoic acid (EPA) and docosa hexaenoic acid (DHA)
sources	orange roughy etc.	deep-sea shark livers	mackerel, tuna, sardines, anchovies and other fish
uses	• industrial degreaser (treatment for industrial dermatitis) • metal cutting, tapping and drilling fluid • as an emollient in cosmetics (face and hand creams, sun creams, lipsticks) • wood and leather treatment	*Squalene* in capsule form as a general health tonic and anti-oxidant, particularly in Asian medicine *DAGE* bolsters immune system	• reduces the incidence of coronary stroke and heart disease in humans • lowers cholesterol • DHA in particular a vital component of brain cell development in infants
markets	United States, Europe, Australia and Asia	Korea, China, Taiwan, Indonesia, Vietnam, Japan and Thailand	Europe, United States, Japan, Korea, Taiwan and China

- have anti-coagulant properties, thereby helping to prevent blood clots from forming too readily and so possible reducing the risk of heart attack;
- reduce the effects of inflammation and may therefore be useful in combating arthritis and rheumatism; and
- reduce blood pressure and potentially protect against heart rhythm disturbances.

Capsules of fish oils containing high levels of the essential eicosapentaenoic acid and docosahexaenoic acid (omega-3 fatty acids) are marketed aggressively internationally, and have captured a small market in Australia. Cudmore says Japan will be the major target market for Beku's omega-3 oils in the next 18 months.

Shark liver oils also can contain significant amounts of diacylglycerol ethers (DAGE). Australian industry presently imports these compounds from overseas, so there is potential for import replacement, and export markets are being developed into Europe, the US and Asia. A range of applications for the DAGE oils are being examined with particular emphasis on their activity in bolstering the human body's immune system and the implications of this for cancer treatment.

Cudmore says Beku's close association with CSIRO has been invaluable in the company's efforts to turn waste from the fishing industry into high value-added products, and in building a highly respected business profile overseas.

Another factor working in Beku's favour is its southern location. Australian marine oils have a natural advantage over those produced in the Northern Hemisphere, many of which contain traces of organic contaminants such as organochlorines. A recent Greenpeace report stated that traces of contaminants have been found in some health products deriving their oils from the north sea region.

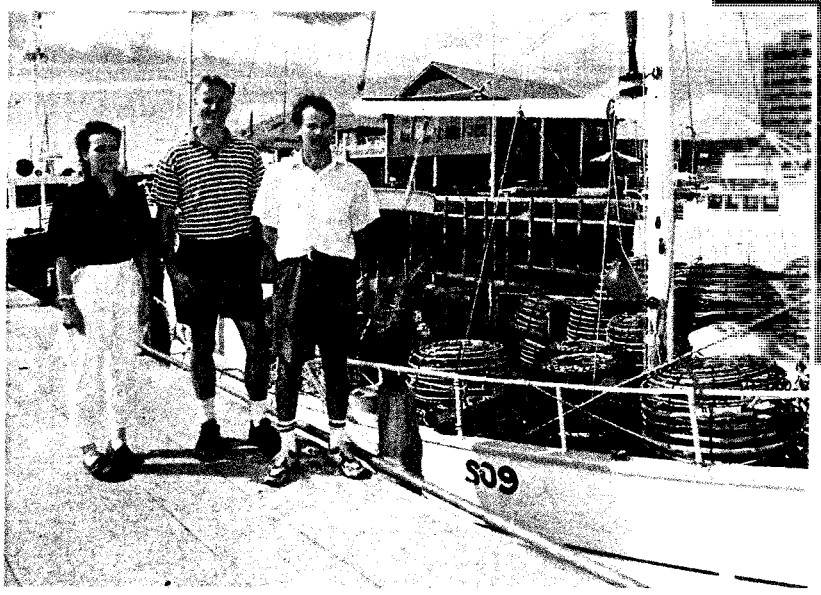
Luckily, the seas around Australia can still be said to have a 'clean green' image. This is partly because the circulation in the Southern Ocean and adjoining seas helps disperse any contaminants over a wide area. Continued research by CSIRO will help Australian industries to develop new marine-oil products that benefit from this natural advantage.

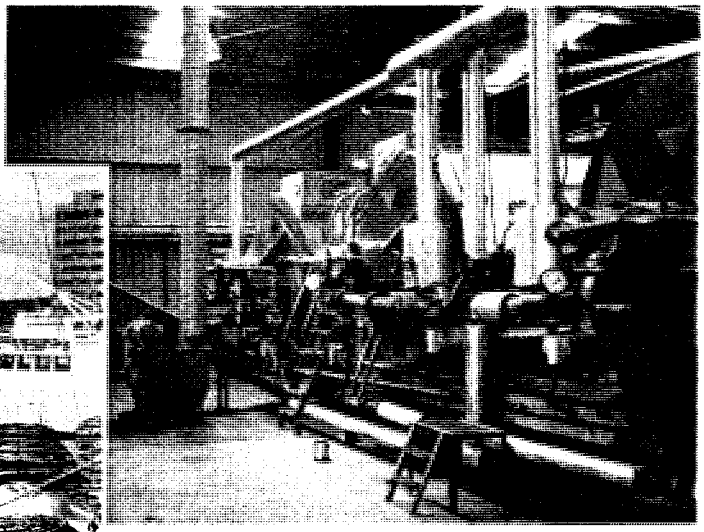
More about marine oils

Davenport SR and Deprez PP (1989) Market opportunities for shark liver oil. *Australian Fisheries* 48:8-10.

Kinsella JE (1987) *Seafoods and fish oils in human health and disease.* Marcel Dekker Inc.

Nichols PD Nichols DS and Bakes MJ (1994) Marine oil products in Australia. *INFORM*:254-261. American Oil Chemists Society.





Above: A fish meal and oil processing factory. Left: Through the Marine Products Project, Dr Patti Virtue, Dr Peter Nichols and Dr Nick Elliott are applying their research skills to help develop a new fishing by-products enterprise.

Lipids and Trophodynamics of Antarctic Zooplankton

by

Charles F. Phleger*

Peter D. Nichols[1] and Patti Virtue[1]

[1]CSIRO Division of Marine Research and Antarctic Co-operative Research Centre

Hobart, Tasmania, 7001, Australia, and the

*Department of Biology, San Diego State University,

San Diego, CA. USA 92182

* To whom correspondence should be addressed at the

Department of Biology
San Diego State University
San Diego, CA 92182
(619) 594-6322
Fax: (619) 594-5676
email: phleger@sunstroke.sdsu.edu

ABSTRACT

Zooplankton were collected by trawl from the Elephant Island region of the Antarctic Peninsula, and from East Antarctica near 63_ - 65_S and 139_ - 150_W. Most zooplankton had low percentages of wax esters (0.0%-7.5%, as percent of total lipid), with the exception of *Thysanoessa macrura* (33.9% wax esters). High triacylglycerol levels were found in *Themisto gaudichaudii* (67.8%, as percent of total lipid), *Euphausia tricantha*, and *E. frigida* (27.2% - 54.2% triacylglycerol), and *Periphylla periphylla* (42.4% - 47.7% triacylglycerol). Polyunsaturated fatty acids (PUFA) were 22.8% - 60.2% of the total fatty acids, with the omega-3 fatty acids eicosapentaenoic acid [20:5(n-3)] and docosahexaenoic acid [22:6(n-3)] being most abundant. The scyphomedusan, *P. periphylla*, was an exception with 12.1% - 19.4% docosapentaenoic acid [22:5 (n-3)] being the major PUFA. The major euphausiid sterols included cholesterol (75% - 92%, as percent of total sterols) and desmosterol (6% - 22%). The major sterols of other zooplankton species were more diverse and included trans-dehydrocholesterol, 24-methylenecholesterol, brassicasterol, and 24-nordehydrocholesterol. The benthic ascidian, *Distaplia cylindrica*, had 45% stanols, as percent of total sterols, whereas the pelagic ascidian *Salpa thompsonii* had only 8% - 11% stanols. Trophodynamic implications of the lipid, fatty acid, and sterol data include an ability to distinguish herbivorous and carnivorous diets and determine survival and reproductive strategies.

INTRODUCTION

Lipid composition can be used to understand and identify food web interactions (Reinhardt and Van Vleet, 1984; Sargent *et al.*, 1987; Graeve *et al.*, 1997). The Southern Ocean has a complex food web including planktivorous herbivores (krill, salps, copepods) fed upon by birds, fish, squid, seals, and baleen whales (Quetin and Ross, 1991). Krill (*Euphausia superba*) provide 30% - 90% of the diet for these carnivores, and have an estimated standing stock biomass of 200 - 400 million metric tons. This high biomass reflects krill's ability to adapt to marked seasonality in food supply. Krill are primarily herbivores feeding on phytoplankton in the summer. In the winter krill feed on ice algae and possibly bacteria and marine snow in addition to using their own body protein as a source of metabolic energy (Virtue *et al.*, 1996). Krill recruitment is highly variable and recruitment is high and spawning early during years when the pack ice concentration is high and of long duration (Siegel and Loeb, 1995). In contrast salp density is low after these cold winters. There is substantial literature on krill lipids (Virtue *et al.*, 1993, 1996; Pond *et al.*, 1995; Saether *et al.*, 1985) because of their commercial interest and undisputed importance in the Southern Ocean. However gelatinous organisms, such as salps, ctenophores, and medusae are often neglected in studies of energy flow through marine pelagic ecosystems. Their importance has only recently been recognized (Longhurst, 1985; Thuesen and Childress, 1994).

The purpose of this study was therefore to clarify and further understand food web interactions and seasonal variability by examining lipids, fatty acids, and sterols as key biochemical factors. Lipids have been used as an investigative tool in this study because they are known to be particularly useful as biochemical probes in biological systems. Lipids are important energy reserve molecules as well as being necessary for cell membrane structure and function, particularly the

polyunsaturated fatty acids, which are required for reproduction and growth (Pond *et al.*, 1995; Phleger, 1991). During the survey by the US Antarctic Marine Living Resources in the region of Elephant Island of the Antarctic Peninsula, and Expedition Broke in Eastern Antarctica, midwater to surface net samples were taken at specific oceanographic stations. From these net samples, zooplankton specimens were taken for the lipid compositonal study reported here.

MATERIALS AND METHODS

Sample Description

Most zooplankton were collected by Isaacs-Kidd Midwater trawl from the R/V YUZHMOREGEOLOGIA during January and February, 1996. The samples were obtained as part of the Antarctic Marine Life Research (AMLR) Field study conducted annually in the Elephant Island region of the Antarctic Peninsula (Martin, 1996; Hewitt and Demer, 1991). The AMLR study area is located between 60-62.5_S and 53-59_W. The net was routinely towed to 170 m depth for about 30 minutes. Benthic ascidians were collected by hand net from the surface, after they had broken loose from the bottom. The three *Periphylla periphylla* samples were taken from one large individual collected in the trawl. These samples were frozen at -10_C as soon as possible after sorting and counting on board ship. They were then transported frozen by air to CSIRO Division of Marine Research, in Hobart, Tasmania, where they were maintained at -60_C prior to analysis. *Euphausia tricantha, E. frigida, E. superba* (males, female, and juvenile) and two of the three *Salpa thompsonii* samples were collected by rectangular midwater trawl (to 195 m) from the R/V AURORA AUSTRALIS on "Expedition Broke" during February and March, 1996, from East Antarctica (Station 181 at 63.4_S, 139_W, and Station 216 at 65_S, 150_W). They were frozen in liquid nitrogen immediately and transported frozen to CSIRO Division of Marine Research for

analysis. *Euphausia superba*, collected in September, 1995, were obtained by MOCNESS from the R/V NATHANIEL PALMER near Elephant Island (61_ 17'S, 55_ 1'W) by Matthew Binder. They were also frozen immediately in liquid nitrogen and maintained at -80_C until analysis.

Lipid Extraction

Samples were quantitatively extracted using a modified Bligh and Dyer (1959) one-phase methanol: chloroform: water extraction (2:1:0.8 v/v/v); samples were extracted overnight and the phases were separated the following day by the addition of chloroform and water (final solvent ratio, 1:1:0.9 v/v/v methanol: chloroform: water). The total solvent extract (TSE) was concentrated (solvents removed *in vacuo*) using rotary evaporation at 30°C. All samples were made up to a known volume in chloroform and stored at -20_C. Samples were stored for up to three days prior to lipid analysis. Samples of all *Euphausia* species, *Tomopteris carpenteri, Themisto gaudichaudii, Sagitta gazellae, Thysanoessa macrura* and *Salpa thompsonii* include three or more pooled individuals for lipid extraction. Single individuals, all that were available to us, were extracted for the rest of the samples.

Lipid Classes

An aliquot of the TSE was analyzed using an Iatroscan MK V TH10 TLC-FID analyzer to determine the abundances of individual lipid classes (Volkman and Nichols, 1991). Each zooplankton sample was applied in duplicate or triplicate to silica gel SIII Chromarods (5 μm particle size) using 1 μL disposable micropipettes. Chromarods were developed in a glass tank lined with pre-extracted filter paper. The solvent system used for the lipid separation was hexane-diethyl ether-acetic acid (60:17:0.2 v/v/v), a mobile phase resolving non-polar compounds such as wax esters, sterol esters, and triacylglycerols, free fatty acids and sterols. A second non-polar solvent system of hexane-diethyl

ether (96:4 v/v) was also used for all samples to resolve hydrocarbons wax esters, triacylglycerols and diacylglyceryl ethers. After development, the chromarods were oven dried and analyzed immediately to minimize adsorption of atmospheric contaminants. The flame ionization detector (FID) was calibrated for each compound class (phosphatidylcholine, cholesterol, cholesterol oleate, oleic acid, squalene, triolein and diacylglyceryl ether, the latter purified from shark liver oil; 0.1 - 10 µg range). Peaks were quantified on an IBM compatible computer using DAPA software (Kalamunda, Western Australia). Iatroscan results are generally reproducible to ±10% or better (Volkman and Nichols, 1991).

Fatty Acids

An aliquot of the TSE was treated with methanol-hydrochloric acid chloroform under nitrogen (10:1:1 v/v/v; 80_C, 2 hr) to form fatty acid methyl esters (FAME). Following the addition of water, products were extracted into hexane/chloroform (4:1 v/v, 3 x 1.5 ml), transferred to vials, reduced under a stream of nitrogen and stored in chloroform.

Gas chromatographic (GC) analyses were performed with a Hewlett Packard 5890A GC equipped with an HP-1 cross-linked methyl silicone fused silica capillary column (50 m x 0.32 mm i.d.), an FID, a split/splitless injector and an HP 7673A auto sampler. Hydrogen was the carrier gas. Following addition of a methyl tricosanoate internal standard, samples were injected in splitless mode at an oven temperature of 50°C. After 1 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 5_C/min. Peaks were quantified with DAPA Scientific Software. Individual components were identified using GC-MS data (see below) and by comparing retention time data with those obtained for authentic and laboratory standards. GC results are subject to an error of ±5%.

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

Determination of Double Bond Configuration in Fatty Acids

Dimethyl disulfide (DMDS) adducts of monounsaturated fatty acids (as methyl esters) were formed by treating the total fatty acid 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

Lipid Composition

Most zooplankton were characterized by low percentages of wax esters (WE) (Table 1). *Thysanoessa macrura* was an exception with 33.9% WE, as percent of total lipid. *Euphausia tricantha* had 5.3% - 7.5% WE in contrast to *E. frigida* with 0.0% WE and *E. superba* with 0.0% - 0.7% WE (Table 1). Two syphomedusae, *Calycopsis borchgrevinki* and *Periphylla periphylla*, had 6.7% WE and 2.6% - 4.5% WE respectively. The rest of the zooplankton had 0.0% - 2.1% WE (Table 1).

Five zooplankton species were characterized by relatively high percentages of triacylglycerol (TAG) (Table 1). The highest TAG level was in *Themisto gaudichaudii* (67.8% of total lipid), which also had 17.7 mg lipid/g (wet weight). *Euphausia tricantha* had 43.4% - 54.2% TAG and *E. frigida* 27.2% TAG. *Euphausia superba*, collected in September, 1995, had 33.2% - 33.4% TAG. Most euphausiids were characterized by lipid levels of 6.4 - 25.1 mg lipid/g wet weight (Table 1). Lipid levels in *E. tricantha* were lower than other euphausiids; 2.5 - 3.2 mg/g. *Periphylla periphylla* had 42.4% - 47.7% TAG, whereas other cnidaria, *Calycopsis borchgrevinki* and *Arctopodema ampla,* had less TAG; 6.9% and 0.4% respectively.

High free fatty acid (FFA) levels (66.7%) were also found in one specimen of *Salpa thompsonii* from the AMLR study area, whereas the eastern Antarctica *S. thompsonii* had 0.0% FFA (Table 1). Other zooplankton species characterized by relatively high FFA content included *Vanadis antarctica* (17.4%), *Tomopteris carpenteri* (25.9%), *Themisto gaudichaudii* (14.0%) *Calycopsis borchgrevinki* (13.2%), *Sagitta gazellae* (31.1%), and *Thysanoessa macrura* (24.2%). These specimens were sorted, counted, and identified in a warm zooplankton laboratory for about 30

minutes prior to freezing at -10_C. The eastern Antarctica *S. thompsonii* with 0.0% FFA, were frozen immediately at -80_C. This was also the case for the September, 1995, *E. superba* as well as *E. tricantha* and *E. frigida*, all characterized by low levels of FFA (Table 1). Phospholipids (PL) comprised 96.9% - 97.8% of total lipid in eastern Antarctica salps, whereas PL was 29.6% in the Elephant Island salps, reflecting the 66.7% FFA. In zooplankton samples that were frozen soon after collection, the FFA were generally low (0.0% - 12.8%, Table 1).

Phospholipids (PL) were the major lipid class in most of the gelatinous zooplankton, comprising 65.4% - 66.9% of *Tomopteris carpenteri* and *Vanadis antarctica*, 62.6% - 74.1% in *Calycopsis borchgrevinki* and *Arctopodema ampla*, 82.8% in *Beroe cucumis*, 96.9% - 97.8% in *Salpa thompsonii* (two specimens), and 59.8% in *Sagitta gazellae*. *Distaplia cylindrica*, a non-gelatinous benthic tunicate had 74.7% PL (Table 1).

Vanadis antarctica and *T. carpenteri* were also characterized by higher lipid content (11.7 - 13.9 mg/g dry weight) in contrast to other gelatinous zooplankton (0.2 - 1.2 mg lipid/g wet weight, Table 1). *Periphylla periphylla* had less PL than the other gelatinous zooplankton (40.3% - 45.2%). Sterols comprised 0.9% - 9.8% in all zooplankton except *V. antarctica* (13.7%) and *A. ampla* (16.9%). Diacylglyceryl ethers (DAGE) were found to be present in two specimens of *P. periphylla* (2.4% - 2.7%), but absent in all other zooplankton.

Sterols

Cholesterol (cholest-5-en-3β-ol) comprised more than 50% of the sterols in seven of the eleven zooplankton species in Table 2 (55.6% - 84.1% of the total sterols). The level of cholesterol in *Thysanoessa macrura* (84.1%) was similar to cholesterol levels in other krill species (82.9% - 100%, Table 3). Trans-dehydrocholesterol (cholesta-5, 22E-dien-3β-ol) comprised 10.5% - 21.9%

of the sterols in the cholesterol-rich zooplankton except for *T. macrura* (0.0%, Table 2). Desmosterol (cholesta-5, 24E-dien-3β-ol) percentages were 1.8% - 20.3% in the cholesterol-rich zooplankton, except for *Calycopsis borchgrevinki* (0.00%) and *Periphylla periphylla* (0.0% - 0.1%) (Table 2). Desmosterol was the second most important sterol in krill, comprising 2.8% -14.4% of the total sterols in all krill species (Table 3).

Zooplankton species with lower cholesterol levels included *Tomopteris carpenteri* (22.5% cholesterol, *Distaplia cylindrica* (5.5% cholesterol), *Arctopodema ampla* (29.2% cholesterol), and *Salpa thompsonii* (6.3% - 14.4% cholesterol) (Table 2). These four zooplankton species were characterized by a broader spectrum of sterols than the cholesterol-rich species, which included as principal components 24-nordehydrocholesterol (24-norcholesta-5,22E-dien-3β-ol) (4.3% -13.3%), transdehydrocholesterol (4.3% - 23.1%), desmosterol (0.4% - 10.9%), brassicasterol (24-methylcholesta-5,22E-dien-3β-ol) (9.2% - 23.7%) and 24-methylenecholesterol (24-methylcholesta-5,24(28)E-dien-3β-ol) (3.8% -19.9%) (Table 2).

The benthic ascidian, *Distaplia cylindrica* had a high percentage of stanols (48.9%), compared to the pelagic ascidian, *Salpa thompsonii*, with 7.9% - 11.2% stanols (Table 2). The principal stanols in *D. cylindrica* included cholestanol (5a-cholestan-3β-ol) (19.5%) 24-nordehydrocholestanol (5a-24-norcholest-22E-en-3β-ol) (5.8%), dehydrocholestanol (5a-cholest-22E-en-3β-ol) (7.0%), brassicastanol (24-methyl-5a-cholest-22E-en-3β-ol) (6.3%), 24-methylenecholestanol (24-methyl-5a-cholest-24(28)E-en-3β-ol) (6.0%), and 24-ethylcholestanol (24-ethyl-5a-cholestan-3β-ol) (3.0%) (Table 2). All other zooplankton were characterized by low stanol content (2.1% and 9.4% total stanols), with *Thysanoessa macrura* having the lowest stanol content (0.6% stanols). The stanol composition of krill (Table 3) was also very low.

Fatty Acids

Most zooplankton were characterized by high levels of omega-3 fatty acids. The sum of the polyunsaturated fatty acids (PUFA) for all zooplankton in Table 4, ranged from 27.8% - 60.2% of the total fatty acids. Eicosapentaenoic acid [EPA, 20:5(n-3)] and docosahexaenoic acid [DHA, 22:6(n-3)] were the two major PUFA's, except in *Periphylla periphylla*, which had 12.1% - 19.4% docosapentaenoic acid [DPA, 22:5(n-3)] in two samples (Table 4). *Periphylla periphylla* had correspondingly low levels of DHA (1.0% - 3.4%) compared with other zooplankton (14.3% - 30.8%). The other cnidaria analyzed had less DPA than *P. periphylla*, and included *Calycopsis borchgrevinki* (5.5%) and *Arctopodema ampla* (0.2%). Highest PUFA levels (over 50% of the total fatty acids) were found in *Tomopteris carpenteri* (58.5% PUFA), *Themisto gaudichaudii* (54.1% PUFA), *Beroe cucumis* (50.1% PUFA), and *Salpa thompsonii* (57.8% - 60.2% PUFA). Most krill samples had fairly high levels of the omega-3 fatty acids also dominated by EPA and DHA, but only one *Euphausia superba* sample (mixed adults) had PUFA levels over 50% of the total fatty acids (58.7%, Table 5). Other omega-3 PUFA were present in lesser amounts than EPA and DHA and included 18:3(n-3) (0.0.% - 1.3%), 18:4(n-3) (0.0% - 8.0%) and 20:4(n-3) (0.0% - 1.5%) (Tables 4, 5).

Monounsaturated fatty acids (MUFA) ranged from 10.7% - 53.4% of the total fatty acids (Tables 4, 5). The major MUFA included 18:1(n-9)c, 18:1(n-7)c, 16:1(n-7)c, 20:1(n-9)c, and 20:1(n-7)c. The MUFA levels in *Euphausia tricantha* (50.3%) and *E. frigida* (38.2% - 48.6%) were higher than for *E. superba*, due primarily to more 18:1(n-9)c (15.3% - 18.5%) than *E. superba* (8.4% - 13.8%) (Table 5). The sum of the MUFA in the polychaete *Vanadis antarctica* (53.4%) was five times greater than in the polychaete *Tomopteris carpenteri* (10.7%), due to increased

vacennic and oleic acids [18:1(n-7)c, and 18:1(n-9)c; 39% in *V. antarctica* and 4.7% in *T. carpenteri;* Table 4].

Percentages of saturated fatty acids (SFA) in the southern ocean zooplankton were lower than unsaturated fatty acids (12.4% - 29.8% of the total fatty acids, Table 4), except for *Thysanoessa macrura* which had 43.0% SFA. This was due primarily to elevated levels of myristic acid (14:0, 19.9%) in *T. macrura* compared to other zooplankton species which had 1.0% - 7.4% of 14:0. *Thysanoessa macrura* also had a somewhat greater percentage of palmitic acid (16:0, 19.3%) compared to the other zooplankton with 5.3% - 16.3% of 16:0 (Table 4). Stearic acid (18:0) ranged from 0.7% - 7.8% in all zooplankton except *Periphylla periphylla* which had 7.0-21.5% of 18:0.

The SFA were generally low in krill (19.2% - 27.9% of the total fatty acids) except for the September, 1995, adults (36.0% - 47.8% SFA, Table 5). These September, 1995, adult krill (*Euphausia superba*) had 16.0% of stearic acid (18:0) compared to 0.9% - 5.9% of 18:0 in the other krill species. Myristic acid (14:0) was high in the other September, 1995, krill sample (10.2%), whereas it was 0.6% - 7.4% in other krill species (Table 5).

DISCUSSION

Lipids in Krill

Lipid levels varied between krill species and between sexes for *Euphausia superba*. Lipid levels in the early spring samples (September), a time of minimal phytoplankton production, were in the same range (24.3 mg/g wet weight) as the female and juvenile summer samples (February) (18.2 -29.5 mg/g (Table 1), a time when phytoplankton production is at its maximum. The September samples were adults which most likely had recently arrived into the Elephant Island area from Bransfield Straight where they presumably had been feeding. Queten *et al.* (1994) also reported higher lipid levels in krill caught in early spring than in krill caught in January. This further emphasizes the fact the lipids in *E. superba* are not used as an overwintering energy source.

Adult male *Euphausia superba* had very low lipid (4.6 mg/g) and triacylglycerol levels (1.7%) compared to females and juveniles (Table 1). These samples were taken in mid summer when phytoplankton was abundant, hence low storage lipid levels may have been the result of other energetic costs. This marked difference in lipid levels between krill sexes has been previously reported by Virtue *et al.* (1996) and low levels of storage lipid in male krill was attributed to reproductive costs associated with mating. Relative levels of polyunsaturated fatty acids (PUFA) were substantially higher in male krill (65.3%) compared to female and juvenile krill (41.1 - 42.1% (Table 5). Most of the PUFA in krill is found in the polar lipid fraction (Virtue *et al.*, 1993). Hence this increase in relative levels of PUFA and lower levels of saturated and monounsaturated fatty acids is consistent with the observed depletion of triacylglycerol and corresponding increase in polar lipid that occurs in male krill.

Euphausia tricantha had much lower lipid levels (2.5 - 3.2 mg/g wet weight) (Table 1). This meso-bathypelagic euphausiid is predominantly carnivorous with large setae mesh size compared to *Euphausia superba.* They are found down to 2500 m and inhabit the warmer waters north of the divergence (north of 63_S) with their distribution being directly correlated with temperature in the east Antarctic region. Being carnivorous this species does not have to store any form of lipid and hence has lower lipid levels compared to the *E. superba* and the omnivore *Euphausia frigida.* The levels of wax esters in *E. tricantha* would indicate that they have been feeding on copepods as some species have high levels of this lipid class (Hagen, 1988). Stomach contents of this species collected at the same time in the same area as the sample analyzed in this study were found to consist exclusively of crustacean parts. This species also had high levels of triacylglycerol which probably indicates that in addition to copepods their diet consists of other species of crustacean and crustacean larvae.

Euphausia frigida is an omnivore and like *Euphausia superba* is a surface water feeder. In the Eastern Antarctic they inhabit waters north of 65_S but tend to be found south of the areas where *Euphausia tricantha* reside. The total lipid in the *E. frigida* sample was 15.8 mg/g wet weight which is higher than *E. tricantha* yet lower than the female and juvenile *E. superba.* The lipid class profile of *E. frigida* was made up of mainly polar lipid (69.1%) which is the structural component of the cell (Table 1). Triacylglycerol levels were lower than that found in *E. tricantha* and female and juvenile *E. superba.* Triacylglycerol is a storage lipid so lower levels infers a reduced feeding activity during that period.

Wax esters

Although most zooplankton were characterized by low levels of WE (0.0% - 2.1%, as percent total lipid), the euphausiid *Thysanoessa macrura* had 33.9% WE (Table 1). Moderate levels of WE (5.3% - 7.5%) were found in another euphausiid, *Euphausia tricantha*, whereas krill, *E. superba* and *E. frigida* either lacked or contained very low levels of WE (0.0% - 0.7%, Table 1; see also Virtue *et al.*, 1993). Adults and larvae of *E. superba* and *E. tricantha* may overwinter by feeding on algae on the underside of pack ice (Siegel and Loeb, 1995; Kawaguchi and Satake, 1994; Loeb *et al.*, 1997). The high WE in *T. macrura* must serve as winter energy reserves since *T. macrura* does not feed on ice algae, and is more carnivorous (Nordhausen, 1994; Mayzaud *et al.*, 1985). Polar copepods are known to synthesize wax esters (Sargent and Henderson, 1986). Low levels of wax esters may indicate a dietary input from copepods, as has been suggested for Arctic benthos (Graeve *et al.*, 1997). The myctophid *Electrona antarctica*, which is the most abundant myctophid in the AMLR study area has 85% - 91% WE in its lipid (466 - 585 mg lipid/g dry weight) (Phleger *et al.*, 1997b).

Sterols of Euphausiids

The principle sterols of all Euphausiids analyzed included cholesterol (81% -100% of total sterols) and demosterol (2% - 17%) (Table 3). According to Goad (1978), crustaceans are incapable of *de novo* sterol synthesis and depend on diet and dealkylation of phytosterols. Desmosterol is produced as an intermediate from phytosterol dealkylation and is also found in marine microalgae *Nitzschia closterium* (100% desmosterol) and *Rhizoselenia setigera* (94.2% desmosterol) (Barret *et al.*, 1995). The waters where some of these krill were collected, known as Bransfield Strait waters, are dominated by microplanktonic diatoms, such as *Chaetoceros, Nitzschia*, and *Rhizoselenia* (Villafañe *et al.*, 1995). The higher cholesterol level in *E. frigida* (92%, Table 3) and

lower desmosterol level (6%) may indicate less recent feeding activity or more efficient phytosterol dealkylation.

Lipids of Cnidaria

Periphylla periphylla and the two hydromedusae *C. borchgrevinki* and *A. ampla* contained low levels of lipid (0.5 - 1.1 mg lipid/g wet weight, Table 1). The low lipid levels in these hydromedusae probably reflects their diet, *Limacina helicina*, which is also low in lipid (Phleger *et al.*, 1997a). In the Arctic, gelatinous zooplankton eat lipid-rich copepods (*Calanus* spp.) and have lipid levels nearly three times higher than Antarctic gelatinous zooplankton (Larson and Harbison, 1989). Although *P. periphylla* contained low absolute levels of lipids, it was characterized by a high relative proportion of TAG (42.4% - 47.7%) compared to 0.4% - 6.9% TAG in the hydromedusae (Table 1). High levels of TAG would normally indicate a lipid-rich diet. The TAG could provide some buoyancy, but buoyancy in gelatinous zooplankton is thought to be achieved primarily by elimination of the sulfate ion (Denton and Shaw, 1961).

High levels of phospholipid (PL) in jelly fish, particularly in the ctenophore *Beroe cucumis* (82.8% PL; Table 1), reflect the importance of membrane lipids as opposed to energy storage lipid (TAG) in these watery zooplankton. *Periphylla periphylla* is an exception with less PL (40.3% - 45.2%, Table 1), reflecting relatively more TAG, as noted above.

The syphomedusae *Calycopsis borchgrevinki* and *Periphylla periphylla* were the only other zooplankton with WE (6.7% WE, and 2.6% - 4.5% WE respectively , Table 1). *Periphylla periphylla* is reported to feed upon copepods and myctophid fishes (V. Loeb, personal communication), and *C. borchgrevinki* may also feed on copepods.

Sterols of the jellies (*C. borchgrevinki, B. cucumis, A. ampla,* and *P. periphylla*) were more diverse than the euphausiids, with 11% - 23% transdehydrocholesterol, 1% - 8% 24-methylenecholesterol, 0% - 9% brassicasterol, and 2% - 10% 24-nordehydrocholesterol also present (Table 2). Transdehydrocholesterol is an intermediate in cholesterol synthesis, and also is found in the hyperiid amphipod *Themisto gaudichaudii* (15%) which eats jellies. Brassicasterol is a major sterol in some Prymnesiophytes, such as *Phaeocystis*, and the sterol 24-methylenecholesterol is the main sterol in the diatom *Chaetoceros* (Tsitsa-Tzardis *et al.*, 1993). Cholesterol was low (29%) in *Arctopodema* which had the most diverse sterol composition. The other jellies had 65% - 66% cholesterol. It is uncertain if Cnidaria can synthesize cholesterol; it may be a slow process and be inhibited by dietary sterols (Goad, 1978).

Lipids of an Hyperiid Amphipod

Themisto gaudichaudii had the highest TAG content (67.8% of total lipid) of the zooplankton analyzed. Since *T. gaudichaudii*, an hyperiid amphipod, feeds on salps and jellyfish, the TAG may help in overwintering beneath the Antarctic ice when salp populations are low. Conversely, it may simply reflect recent feeding activity. The PUFA content of *T. gaudichaudii* (54.1% of total fatty acids, Table 4) includes 20.4% of 22:6(n-3) and 24.1% of 20:5(n-3). These are higher values than those reported by Fricke and Oehlenschläger (1988) for *Themisto*, and may reflect different food sources. *Themisto* also has 15% transdehydrocholesterol (Table 4) which may be derived from its jellyfish diet.

Lipids of Polychaetes

The polychaetes *Vanadis antarctica* and *Tomopteris carpenteri* had lipid consisting primarily of PL (65.4% - 66.9%, Table 1) which agrees with Hagen (1988). The lipid content of these

polychaetes (11.7 - 13.9 mg/g wet weight) was lower than those reported by Hagen (1988) (9% - 17% lipid, as percent dry weight). The PUFA content in *V. antarctica* (37.9% of total fatty acids) is less than that of *T. carpenteri* (58.5%) due to twice as much 22:6(n-3) in *T. carpenteri* (Table 4). *Tomopteris* has less cholesterol (23%) than *V. antarctica* (58%) (Table 1). *Tomopteris* eats much smaller organisms than *Vanadis*, which may prey upon larval fish, resulting in the higher cholesterol value. *Tomopteris carpenteri* has higher levels of 24-nordehydrocholesterol (10% of total sterols), brassicasterol (11%), and 24-methylenecholesterol (12%) than *V. antarctica* (Table 2). This also may reflect a different diet. Sterols are useful indicators of dietary composition in marine herbivores (Virtue *et al.*, 1993). Transdehydrocholesterol comprised 22% -23% of the polychaete sterols (Table 2). Transdehydrocholesterol values (22% - 23%) were the same in the hydromedusae, *Calycopsis borchgrevinki* and *Arctopodema ampla*.

Diacylglyceryl ethers

Two of the *P. periphylla* samples had low levels of diacylglyceryl ether (DAGE) (2.4% - 2.7%, of total lipid). DAGE was not detected in any other zooplankton samples . DAGE have been reported in low levels (0.3% - 0.6%) in *E. superba* (Fricke *et al.*, 1986). Recently, however, 28% DAGE has been reported in another Antarctic zooplankton, the gymnosome pteropod *Clione limacina* (Phleger *et al.*, 1997a) where a buoyancy role for this lipid class was proposed. *Spongiobranchaea australis*, another Antarctic gymnosome pteropod, has 0.9% - 1.7% DAGE, but less lipid (3.3 - 4.8 mg lipid/g wet weight) than *Clione* (50.8 mg lipid/g wet weight) (Phleger *et al.*, 1997a). *Periphylla periphylla* might obtain DAGE from these pteropods, which could comprise part of its diet , or from the low levels reported in krill.

Lipids of Ascidians and Sagitta

The lipids of *Salpa thompsonii, Distaplia cylindrica* and *Sagitta gazellae* (Table 1), reflect different ecological positions and dietary preferences. *Sagitta gazellae*, contains relatively low levels of energy-storage lipid (9.9% TAG) and has relatively high levels of phospholipid (PL) (59.8%). These results are in agreement with those of Hagen and Piatkowski (1994) and Falk-Petersen *et al.*, (1987) for *Sagitta* sp. *Sagitta* probably finds enough food throughout the winter months because it is a predaceous carnivore. *Distaplia cylindrica*, a benthic filter-feeder, has 74.7% PL and only 10.6% TAG. It also must overwinter feeding on an opportunistic basis, because it lacks energy-storage lipid. Populations of *Salpa* decrease during winter, which reflects less food availability for these pelagic filter feeders, and their populations increase dramatically in the spring. There is 17% - 19% oleic acid (18:1) in *Sagitta* and *Distaplia*, whereas *Salpa* only has 5% of this monoenic acid. Lower levels of oleic acid in *Salpa* reflects its phytoplankton-rich diet. *Sagitta* and the benthic *Distaplia* have higher levels of 18:1, reflecting their different carnivorous diets. The presence of high levels of stanols in *Distaplia*, such as cholestanol (20%), 24-nordehydrocholestanol (6%), brassicastanol (6%), and 24-methylenecholestanol (6%), may reflect reducing activity (anoxia) in the sediments where they live. Stanols, such as coprostanol (5β-cholestanol) produced in the digestive tract of higher animals by microbial degration of cholesterol, are used as biomarkers in sediments (Nichols *et al.*, 1993). The high stanol levels in *Distaplia* therefore may also result from biohydrogenation and incorporation of dietary components. The sterol profile of *Sagitta* (58% cholesterol, 19% transdehyrocholesterol, 4% nordehydrocholesterol) was similar to that observed in the polychaete *V. antarctica*, although desmosterol was reduced in *Sagitta* (2% cf 10%). As for *V. antarctica* and other zooplankton, the profile is consistent with a carnivorous diet. The sterol profile of *Salpa thompsonii* (19 - 20% cholesterol, 8-15% transdehydrocholesterol, 14-20% 24-

methylenecholesterol , 7-13% 24-nordehydrocholesterol, 19-20% brassicasterol) is as noted for *Tomopteris carpenteri* consistent with an herbivorous diet. The *S. thompsonii* sample from Elep Island had free fatty acid levels (FFA)

of 66.7% (as percent total lipid) compared to 0.0% for the *S. thompsonii* from Eastern Antarctic (Table 1). The elevated FFA levels probably reflect the process of sorting and counting in a heat zooplankton laboratory on board ship for the Elephant Island samples. In contrast, Eastern Antarctica samples were frozen immediately after capture in liquid nitrogen which kept lipids inta Since the PUFA content in the Elephant Island *S thompsonii* sample was high and compares favorably with the Eastern Antarctica PUFA (Table 4), the 30 minute sorting process in the laboratory did not appear to affect the fatty acids.

Conclusions

Trophodynamic implications of the lipid, fatty acid, and sterol data presented here includ an ability to distinguish herbivorous and carnivorous diets and determine survival and reproductiv strategies. Lipids indeed provide a window to look at aspects of trophodynamics not visible by conventional techniques.

ACKNOWLEDGMENTS

This work was supported in part by funding from FRDC. We thank J. Martin and R. Hewitt for the AMLR Expedition to the Elephant Island study area and the Australian National Antarctic Research Expedition for collection of samples in East Antarctica. V. Siegel, D. Outram, V. Loeb, and M. Binder gave invaluable help in collection and identification of samples. C. Phleger thanks the Department of Biology, San Diego State University , for sabbatical leave time to work on the research. Ben Mooney gave technical assistance and Danny Holdsworth managed the CSIRO GC-MS facility.

References

Barrett SM, Volkman JK, Dunstan GA (1995) Sterols of 14 species of marine diatoms (Bacillariophyta). J Phycol 31: 360-369.

Bligh EG, Dyer W (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37: 911-917.1

Denton EJ, Shaw TI (1961) The buoyancy of gelatinous marine animals. J Physiol London 161: 14-15.

Dunkelblum E, Tan SH, Silk RJ (1985) Double-bond location in monounsaturated fatty acids by dimethyl disulphide derivitization and mass spectrometry. J Chem Ecol 11: 265-277.

Dunstan GA, Baillie HJ, Barrett SM, Volkman JK (1996) Effect of diet on the lipid composition of wild and cultured abalone. Aquaculture 140: 115-127.

Falk-Petersen S, Sargent JR, Tande KS (1987) Lipid composition of zooplankton in relation to the sub-Arctic food web. Polar Biol 8: 115-120.

Fricke H, Oehlenschläger J (1988) Fatty acid and sterol composition of the Antarctic Amphipod *Themisto gaudichaudii* Guerin 1828. Comp Biochem Physiol 89B: 39-42.

Fricke H, Gercken G, Oehlenschäger J (1986) 1-0-Alkylglycerol lipids in Antarctic krill (*Euphausia superba* Dana) Comp Biochem Physiol 85B: 131-134.

Goad LJ (1978) The sterols of marine invertebrates: composition, biosynthesis, and metabolites. In: Scheuer PJ (ed) Marine Natural Products: Chemical and Biological Perspectives. Academic Press, New York.

Graeve M, Kattner G, Piepenburg D (1997) Lipids in Arctic benthos: does the fatty acid and alcohol composition reflect feeding and trophic interactions? Polar Biol 18: 53-61.

Hagen W, Piatkowski U (1994) Distribution and lipid composition of chaetognaths in the Weddell Sea, Antarctica. Scar Sixth Biology Symposium, Antarctic Communities: species, Structure, and Survival. Venice. Abstracts. pp. 113.

Hagen W (1988) On the significance of lipids in Antarctic zooplankton. Berichte zur Polar forschung 49: 1-117.

Hewitt RP, Demer DA (1991) Krill abundance. Nature 353:310.

Kawaguchi K, Satake M (1994) Relationship between recruitment of Antarctic krill and the degree of ice cover over the South Shetland Islands. Fisheries Science 60: 123-124.

Larson RJ, Harbison GR (1989) Source and fate of lipids in polar gelatinous zooplankton. Arctic 42: 339-346.

Loeb V, Siegel V, Holm-Hansen O, Hewitt R, Fraser W, Trivelpiece W, Trivelpiece S (1997) Effects of sea ice extent and krill on salp dominance on the Antarctic food web. Nature (in press).

Longhurst AR (1985). The structure and evolution of plankton communities. Prog Oceanog 15: 1-35.

Martin J (1996) AMLR 1995/96 Field Season Report LJ-95. 14, Southwest Fisheries Science Center, Antarctic Ecosystem Research Group.

Mayzaud P, Farber-Lorda J, Corre MC (1985) Aspects of the nutritional metabolism of two Antarctic Euphausiids: *Euphausia superba* and *Thysanoessa macrura.* In: Siegfried WR, Condy PR, Laws RM (eds) Antarctic Nutrient Cycles and Food Webs, Springer-Verlag, Berlin, pp. 330-338.

Nichols PD, Guckert JB, White DC (1986) Determination of monounsaturated fatty acid double-bond position and geometry for microbial monocultures and complex consortia by capillary GC-MS of their dimethyl disulphide adducts. J Microbiol Meth 5: 49-55.

Nichols PD, Leeming R, Rayner MS, Latham V, Ashbolt NJ, Turner C (1993) Comparison of the abundance of the fecal sterol coprostanol and fecal bacterial groups in inner-shelf waters and sediments near Sydney, Australia. J Chromat 643: 198-195.

Nordhausen W (1994) Winter abundance and distribution of *Euphausia superba, E. crystallorophias*, and *Thysanoessa macrura* in Gerlach Strait and Crystal Sound, Antarctica. Mar Ecol Prog Ser 190: 131-142.

Phleger CF, Nichols PD Virtue P (1997a) The lipid, fatty acid and fatty alcohol compositon of the myctophid fish *Electrona antarctica*: High level of wax esters and food-chain implications. Antarct Sci 9: 258-265.

Phleger CF, Nichols PD, Virtue P (1997b) Lipids and buoyancy in southern ocean pteropods. Lipids (in press).

Phleger CF (1991) Biochemical aspects of buoyancy in fishes. In: Hochachka PW, Mommsen TP (eds) Biochemistry and Molecular Biology of Fishes. Vol 1, Phylogenetic and Biochemical Perspectives, Elsevier Science Publishers. B.V. Chap. 9: 209-247.

Pond D, Watkins J, Priddle J, Sargent J (1995) Variation in the lipid content and composition of Antarctic krill *Euphausia superba* at south Georgia. Mar Ecol Progr Ser 117: 49-57.

Quetin LB, Ross RM (1991) Behavioral and physiological characteristics of the Antarctic krill, *Euphausia superba*. Amer Zool 31: 49-63.

Quetin LB, Ross RM, Clark A (1994) Krill energetics; seasonal and environmental aspects of the physiology of *Euphausia superba*. In: El-Sayed, SZ (ed) Southern Ocean Ecology, the BIOMASS Prospective. Cambridge University Press, Cambridge, England, 165-184.

Reinhardt SB, Van Vleet ES (1986) Lipid composition of twenty-two species of Antarctic midwater zooplankton and fish. Mar Biol 91: 149-159.

Reinhardt SB, Van Vleet ES (1984) Lipid composition of Antarctic midwater invertebrates. Antarctic J Review: 139-141.

Saether O, Ellingsen TE, Mohr V (1985) The distribution of lipid in the tissues of Antarctic krill, *Euphausia superba*. Comp Biochem Physiol 818: 609-614.

Sargent, JR, Henderson RJ (1986) Lipids. In: Corner EDS, O'Hara SC (eds) The Biological Chemistry of Marine Copepods. Clarendon Press, Oxford, England.

Sargent JR, Parkes RJ, Mueller-Harvey I, Henderson RJ (1987) Lipid biomarkers in marine ecology. In: Microbes in the sea. Ellis Horwood, Chichester, p. 119-138.

Siegel V, Loeb V (1995) Recruitment of Antarctic krill *Euphausia superba* and possible causes for its variability. Mar Ecol Prog Ser 123: 45-56.

Thuesen EV, Childress JJ (1994) Oxygen consumption rates and metabolic activities of oceanic California medusae in relation to body size and habitat depth. Biol Bull 187: 84-98.

Tsitsa-Tzardis E Patterson GW, Wikfors GH, Gladu PK, Harrison D (1993) Sterols of *Chaetoceros* and *Skeletonema*. Lipids 28: 465-467.

Villafane VE, Helbling EW, Holm-Hansen O (1995) Spatial and temporal variability of phytoplankton biomass and taxonomic composition around Elephant Island, Antarctica, during the summers of 1990-1993. Mar Biol 123: 677-686.

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

Virtue P, Nichols PD, Nicol S, Hosie G (1996) Reproductive trade off in male Antarctic krill. Mar Biol 126: 521-527.

Volkman JK, Nichols PD (1991) Applications of thin layer chromatography-flame ionization detection to the analysis of lipids and pollutants in marine environmental samples. J Planar Chrom 4: 19-26.

Table 1. Composition of Lipids from Southern Ocean Zooplankton

Sample	Percentage Composition									
	WE	TAG	FFA	ST	PL	UN	DAGE	ALC	TOTAL	Lipid content mg/g
Cnidaria										
Periphylla periphylla	4.5	47.7	1.5	5.9	40.4	0.0			100.0	0.6
	2.8	42.4	1.8	5.1	45.2	0.0	2.7		100.0	1.1
	2.6	46.4	3.7	4.6	40.3	0.0	2.4		100.0	0.8
Calycopsis borchgrevinki	6.7	6.9	13.2	9.8	62.6	0.0		0.8	100.0	0.5
Arctopodema ampla	0.4	0.4	7.1	16.9	74.1	1.0		0.0	100.0	0.5
Ctenophora										
Beroe cucumis	2.1	2.3	6.6	3.0	82.8	0.0		3.2	100.0	0.2
Chaetognatha										
Sagitta gazellae	1.1	0.0	31.1	7.2	59.8	0.5		0.3	100.0	1.2
Polychaetes										
Vanadis antarctica	2.0	--	17.4	13.7	66.9	--			100.0	11.7
Tomopteris carpenteri	1.9	--	25.9	6.4	65.4	0.5			100.0	13.9
Hyperiidea										
Themisto gaudichaudi	0.9	67.8	14.0	2.0	15.3	--			100.0	17.7
Ascidians										
Salpa thompsonii	0.0	0.0	66.7	3.7	29.6	0.0			100.0	0.7
EA*	0.0	0.0	0.0	2.2	97.8				100.0	0.9
EA*	0.0	0.0	0.0	3.1	96.9				100.0	0.8
Distaplia cylindrica	1.2	10.6	3.9	9.1	74.7	0.5			100.0	2.7
Euphausiacea										
Euphausia superbas										
Sept 95*	0.2	33.4	12.8	2.2	51.3				100.0	24.3
Sept 95*	0.3	33.2	5.9	0.9	59.7				100.0	ND
Sub Adult Male EA*	0.0	16.5	1.5	4.3	77.7				100.0	18.5
Male EA*	0.0	1.7	0.0	5.4	93. 1				100.0	4.6
Female EA*	0.2	37.3	0.0	4.1	58.2				100.0	29.5
Juvenile EA*	0.0	39.5	0.0	3.7	64.2				100.0	18.2
Euphausia tricantha EA	7.5	54.2	1.0	1.0	36.3				100.0	3.2
EA	5.3	43.7	1.1	1.2	48.6				100.0	2.5
Euphausia frigida EA	0.0	27.2	0.7	3.1	69.1				100.0	15.8
Thysanoessa macrura	33.9	0.0	24.2	0.9	39.6	0.5		0.9	100.0	7.7

WE = Wax ester, TAG = Triacylglycerol, FFA = Free fatty acid, ST = Sterol, PL = Polar lipid, UN = Unidentified, DAGE = Diacylglyceryl ether, ALC = Alcohol

*EA means that these samples were collected in eastern Antarctica

Table 2. Sterol Composition of Southern Ocean Zooplankton

		Percentage Composition													
Sterol	Peak No	*Periphylla periphylla*			*Calycopsis borchgrevinki*	*Artopodema ampla*	*Beroe cucumis*	*Sagitta gazellae*	*Vanadis antarctica*	*Tomopteris carpenteri*	*Themisto gaudichaudii*	*Salpa thompsonii*			*Distaplia cylindrica*
24-nordehydrocholesterol	1	4.0	4.2	4.2	2.7	9.6	1.6	4.3	3.8	10.4	1.5	13.3	7.2	6.5	4.3
24-nordehydrocholestanol	2	0.6	0.6	0.6	0.3	0.6	0.3	0.5	0.0	1.6	0.0	2.4	1.8	0.0	5.8
cis-dehydrocholesterol	3	0.2	1.1	1.6	0.8	2.1	0.4	0.4	0.6	1.5	0.3	2.9	1.6	0.0	2.9
trans-dehydrocholesterol	4	14.7	15.3	14.7	22.1	23.1	10.5	18.8	21.9	22.8	14.8	8.0	15.4	15.4	4.3
dehydrocholestanol	5	1.1	1.2	1.1	0.8	0.7	0.5	1.2	0.3	2.0	0.4	1.5	2.1	2.6	7.0
cholesterol	6	64.8	64.2	64.6	65.9	29.2	64.9	58.4	58.2	22.5	55.6	6.3	13.7	14.4	5.5
cholestanol	7	1.2	1.1	1.3	1.0	0.6	0.9	2.9	0.0	1.2	1.0	1.18	1.2	0.0	19.5
demosterol	8	0.1	0.1	0.0	0.0	10.9	14.9	1.8	10.4	7.6	20.3	4.9	7.2	6.2	0.4
demostanol	9	0.0	0.0	0.0	0.0	0.0	1.6	0.0	1.5	0.0	1.3	0.0	0.0	0.0	0.0
brassicasterol	10	5.3	5.0	4.9	2.5	9.2	0.0	1.6	0.0	10.7	0.0	20.4	20.2	19.0	23.7
brassicastanol	11	1.1	1.1	1.0	0.4	0.9	0.9	0.8	0.0	1.3	0.0	3.3	5.1	5.3	6.3
24-methylenecholesterol	12	3.7	3.7	3.6	1.6	8.4	1.2	1.8	2.7	12.4	3.9	20.0	15.0	13.6	3.8
24-methylenecholestanol	13	1.2	1.2	1.2	0.4	0.9	0.4	0.5	0.3	2.9	0.4	2.3	0.0	0.0	6.0
stigmasterol	14	0.2	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.3	0.0	1.6	1.3	0.0	0.0
stigmastanol	15	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.3	0.0	0.0	0.7
24-ethylcholesterol	16	0.5	0.3	0.3	0.5	1.1	0.4	0.5	0.0	1.2	0.0	3.7	2.3	2.5	3.3
24-ethylcholestanol	17	0.3	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.0
isofucosterol	18	0.5	0.2	0.2	0.0	0.3	0.0	0.0	0.0	1.3	0.0	3.6	2.4	2.2	0.0
isofucostanol	19	0.0	0.0	0.1	0.0	0.2	0.0	0.0	0.0	0.2	0.0	0.3	0.0	0.0	0.7
C30, 2 double bonds	20	0.0	0.0	0.0	1.0	2.4	0.0	4.8	0.0	0.0	0.0	0.0	0.0	0.0	1.0
Other		0.5	0.6	0.4	0.2	0.0	1.1	1.9	0.0	0.0	0.6	0.9	2.7	12.4	2.1
Total		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Table 3. Sterol Composition of Southern Ocean Krill

Sterol	Peak No	September 1995		Percentage Composition: *Euphausia superba* Sub-Male	*Euphausia superba* Male	*Euphausia superba* Adults Female	*Euphausia superba* Adults Juvenile	*Euphausia tricantha*		*Euphausia frigida*	*Thysanoessa macrura*
24-nordehydrocholesterol	1	0.0	0.0	0.0	0.0	0.2	0.3	0.0	0.0	0.0	0.0
cis-dehydrocholesterol	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
trans-dehydrocholesterol	4	0.0	0.6	0.0	0.0	0.0	0.0	1.5	1.3	1.2	0.0
cholesterol	6	86.6	86.3	92.3	100.0	88.4	86.2	82.9	84.3	92.2	84.1
cholestanol	7	0.0	0.2	0.0	0.0	0.0	0.0	1.7	0.0	0.0	0.1
demosterol	8	13.4	11.5	3.5	0.0	2.8	3.5	14.0	14.45	6.2	12.4
demostanol	9	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
brassicasterol	10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	1.0
24-methylenecholesterol	12	0.0	0.0	4.2	0.0	8.7	10.3	0.0	0.0	0.0	0.7
24-ethylcholesterol	16	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
isofucosterol	18	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C30, 2 double bonds	20	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7
Other		0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Table 4. Fatty Acid Composition of Southern Ocean Zooplankton

	Percentage Composition													
Fatty Acid	*Vanadis antarctica*	*Tomopteris carpenteri*	*Distaplia cylindrica*	*Themisto gaudichaudii*	*Calycopsis borchgrevinki*	*Beroe cucumis*	*Artopodema ampla*	*Sagitta gazellae*	*Periphylla periphylla*			*Salpa thompsonii*		
12:0	0.0	0.0	0.0	0.1	0.1	1.9	0.3	0.3	0.1	0.0	0.0	0.1	0.0	0.0
13:0	0.0	0.1	0.0	0.0	0.1	0.1	0.2	0.3	0.0	0.0	0.0	0.1	0.0	0.0
14:0	1.9	7.3	6.4	4.6	.27	7.4	5.5	3.8	3.1	1.0	3.8	6.4	5.3	5.0
15:0	0.2	0.8	1.9	0.6	0.6	0.5	1.2	0.6	0.5	0.2	0.5	1.6	2.4	2.9
16:0	5.3	13.1	14.9	15.0	11.6	14.3	14.9	13.8	13.6	13.5	15.7	16.0	15.4	16.3
17:0	0.2	0.6	1.4	0.3	0.6	0.3	0.5	0.4	0.4	0.8	0.5	0.4	0.5	0.6
18:0	4.4	3.8	4.5	1.0	7.8	3.3	5.1	3.1	7.0	21.5	7.8	1.9	0.7	1.2
19:0	0.0	0.1	0.2	0.1	0.2	0.2	0.3	0.2	0.2	0.3	0.2	0.0	0.0	0.2
20:0	0.0	0.2	0.4	0.1	0.2	0.1	0.3	0.1	0.2	0.6	0.2	0.1	0.1	0.1
22:0	0.4	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
Sum Saturates	12.4	26.1	29.8	21.9	24.1	26.1	28.1	22.4	25.2	37.9	28.9	26.8	24.4	26.3
il4:0	0.0	0.1	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.2	0.0	0.0
il5:0	0.1	0.2	1.1	0.3	0.5	0.2	0.3	0.2	0.2	0.0	0.2	0.4	0.3	0.3
al5:0	0.1	0.1	0.5	0.1	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.0
il6:0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.1	0.2	0.3	0.4
br17:1	0.1	0.3	0.0	0.1	0.9	0.0	0.0	0.1	0.0	0.0	0.0	0.2	0.0	0.0
il7:0	0.3	0.6	1.5	0.4	0.6	0.2	0.4	0.3	0.4	0.3	0.5	0.4	0.4	0.6
al7:0	0.1	0.2	0.5	0.6	0.7	0.2	0.8	1.1	0.3	0.3	0.4	0.5	0.0	0.0
il9:0	0.5	0.1	0.0	0.1	0.1	0.0	0.0	0.1	0.2	0.0	0.0	0.0	0.0	0.0
Sum Branched	1.1	1.6	3.7	1.6	2.9	0.6	1.7	1.9	1.3	0.6	1.3	2.0	1.0	1.3
14:1(n-7)c	0.0	0.1	0.0	0.0	0.1	0.1	0.3	0.0	0.1	0.0	0.0	0.1	0.0	0.0
14:1(n-5)c	0.0	0.0	0.0	0.0	0.1	0.2	0.3	0.3	0.0	0.0	0.0	0.1	0.0	0.0
16:1(n-9)c	0.3	0.1	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0
16:1(n-7)c	3.2	0.9	5.1	4.1	5.1	1.1	2.7	5.5	1.9	0.0	2.2	4.1	4.1	4.1
16:1(n-7)t	0.2	0.2	0.0	0.1	0.1	0.0	0.1	0.0	0.2	0.0	0.2	0.5	0.0	0.0
16:1(n-5)c	0.1	0.2	1.6	0.5	0.0	0.2	0.0	0.4	0.1	0.0	0.1	0.8	0.5	0.4
17:1(n-8)c	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18:1(n-9)c	13.6	2.9	5.6	8.7	14.3	10.4	5.7	11.0	14.3	6.7	15.5	2.5	3.3	3.8
18:1(n-7)c	25.4	1.8	11.7	4.0	1.8	6.4	1.8	6.4	2.5	2.4	3.2	2.8	4.2	4.5
18:l(n-7)t	0.0	0.1	0.3	0.2	0.1	0.0	0.2	0.1	0.0	0.0	0.0	0.3	0.1	0.0
18:1(n-5)c	1.1	0.6	0.6	0.7	1.1	0.6	0.5	1.4	0.7	0.3	0.8	0.7	0.1	0.0
20:1(n-11)+(n-3)c	0.0	0.9	0.3	1.9	0.1	0.1	0.0	0.2	0.1	0.0	0.0	0.2	0.0	0.0
20:1(n-9)c	8.7	3.1	3.7	1.1	4.4	1.9	1.8	1.3	4.7	4.5	4.4	1.0	0.0	0.0

Table 4. continued

Fatty Acid	Percentage Composition														
	Vanadis antarctica	*Tomopteris carpenteri*	*Distaplia cylindrica*	*Themisto gaudichaudii*	*Calycopsis borchgrevinki*	*Beroe cucumis*	*Artopodema ampla*	*Sagitta gazellae*	*Periphylla periphylla*			*Salpa thompsonii*			
20:1(n-7)c	0.2	0.5	2.9	0.1	6.9	1.8	2.0	1.6	5.2	4.7	5.1	0.3	0.0	0.0	
22:1(n-11)c	0.2	0.1	0.0	0.0	0.9	0.0	0.0	0.0	1.9	2.4	1.7	0.0	0.0	0.0	
22:1(n-9)c	0.1	0.5	0.3	0.2	0.3	0.6	2.7	0.3	3.2	4.5	3.3	0.0	0.1	0.1	
22:1(n-7)c	0.0	0.2	0.4	0.1	0.1	0.0	0.1	0.3	0.2	0.2	0.2	0.0	0.0	0.0	
24:1(n-9)c	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.2	0.0	0.0	0.0	
Sum Monounsaturates	53.4	10.7	27.5	21.9	35.3	23.1	18.0	28.6	35.2	25.7	36.9	13.4	12.5	12.9	
C16 PUFA	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	1.7	0.0	0.0	
18:2(n-6)	0.9	0.8	1.3	2.5	0.9	2.2	0.9	1.6	0.9	0.3	1.0	1.9	0.2	0.0	
18:3(n-3)	0.1	0.2	0.8	0.6	0.0	0.4	0.1	0.3	0.1	0.0	0.2	1.0	0.0	0.1	
18:4(n-3)	0.2	1.9	0.0	3.8	0.1	1.1	0.2	0.3	0.6	0.0	0.8	8.0	2.6	1.3	
20:2(n-6)	0.2	1.1	0.9	0.9	0.8	0.7	0.6	0.1	0.7	0.3	0.6	0.7	0.0	0.0	
20:3(n-6)	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.4	0.4	
20:4(n-3)	0.9	1.5	0.9	0.9	0.8	0.7	0.3	0.1	0.6	0.0	0.7	0.5	0.0	0.0	
20:4(n-6)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	
20:5(n-3)	13.4	21.4	20.8	24.0	9.9	18.6	16.4	14.3	17.7	1.5	20.9	19.2	27.5	25.6	
C21PUFA	0.0	0.0	0.0	0.0	0.0	0.0	1.2	0.0	1.4	0.0	0.0	0.0	0.0	0.0	
C22PUFA	1.7	1.0	0.0	0.2	0.4	0.1	0.1	0.0	0.3	0.0	0.4	0.0	0.0	0.0	
C22PUFA	0.6	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
22:4(n-6)	0.3	0.0	0.0	0.0	2.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
22:5(n-3)	0.3	2.2	1.2	0.8	5.6	0.4	0.2	0.3	12.1	19.4	0.5	0.5	0.4	0.4	
22:6(n-3)	14.3	28.2	14.5	20.4	16.1	25.7	26.4	21.3	2.6	1.0	3.4	24.3	29.2	30.8	
Sum PUFA	32.9	58.5	38.2	54.1	36.8	50.1	46.4	38.4	36.9	22.8	28.5	57.8	60.2	58.7	
Other	0.2	3.1	0.9	0.4	0.9	0.1	5.8	8.7	1.4	13.0	4.5	0.0	1.9	0.8	
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	

Table 5. Fatty Acid Composition of Southern Ocean Krill

	Percentage Composition									
			Euphausia superba				*Euphausia tricantha*		*Euphausia frigida*	*Thysanoessa macrura*
Fatty Acid	Sept 1995		Sub-Adult Male	Adults Male	Adults Female	Adults Juvenile				
12:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.5
13:0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
14:0	10.2	1.9	3.2	1.2	7.6	7.4	0.9	0.6	0.9	19.9
15:0	0.4	0.3	0.5	0.0	0.3	0.5	0.3	0.2	0.2	0.3
16:0	24.0	27.4	17.2	16.2	18.5	19.3	12.4	13.4	18.0	19.3
17:0	0.1	0.8	0.0	0.0	0.1	0.2	0.3	0.4	0.4	0.1
18:0	1.2	16.0	1.5	1.2	0.9	3.1	4.9	5.6	5.9	2.4
19:0	0.0	0.3	0.0	0.2	0.0	0.0	0.2	0.0	0.3	0.0
20:0	0.1	0.3	0.3	0.4	0.3	0.1	0.4	0.4	0.4	0.0
22:0	0.1	0.3	0.1	0.2	0.0	0.0	0.2	0.2	0.1	0.0
24:0	0.1	0.3	0.0	0.0	0.0	0.0	0.2	0.2	0.1	0.0
Sum Saturates	**36.0**	**47.8**	**22.8**	**19.2**	**27.9**	**30.6**	**19.6**	**21.2**	**26.3**	**43.0**
il4:0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
il5:0	0.2	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.3
il6:0	0.0	0.1	0.0	0.0	0.0	0.0	0.2	0.2	0.1	0.1
il7:0	0.3	0.3	0.5	0.0	0.2	0.4	0.6	0.5	0.5	0.2
al7:0	0.0	0.0	0.6	0.0	0.3	0.2	0.6	1.5	0.5	0.3
Sum Branched	**0.6**	**0.6**	**1.1**	**0.0**	**0.5**	**0.6**	**1.6**	**2.3**	**1.1**	**1.0**
14:1(n-5)c	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7
16:1(n-7)c	7.9	1.6	0.9	1.3	8.1	1.7	1.7	1.5	2.2	2.1
16:1(n-7)t	0.0	0.1	0.0	0.0	0.0	0.0	0.2	0.1	0.0	0.1
16:1(n-5)c	0.4	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	1.2
18:1(n-9)c	12.0	13.8	0.0	5.9	10.0	10.3	18.5	17.7	15.3	7.3
18:1(n-7)c	8.2	13.0	12.2	8.0	10.9	13.4	10.7	10.5	12.9	3.7
18:1(n-7)t	0.0	0.0	10.6	0.0	0.3	0.0	0.0	0.0	0.0	0.1
18:1(n-5)c	0.2	0.0	0.0	0.0	1.3	0.0	0.8	0.8	0.5	0.3
20:1(n-11)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3
20:1(n-9)c	0.9	2.7	0.4	0.3	0.6	0.7	8.7	9.5	3.1	0.4
20:1(n-7)c	0.4	1.2	0.0	0.0	0.2	0.8	3.6	3.9	1.7	0.1
22:1(n-11)	0.0	0.0	0.0	0.0	0.0	0.0	1.6	1.0	0.3	0.1
22:1(n-9)c	0.7	1.8	0.0	0.0	0.0	0.0	3.2	2.5	1.2	0.0
22:1(n-7)c	0.2	0.6	0.0	0.0	0.0	0.0	0.5	0.5	0.7	0.0
24:1(n-9)c	0.0	0.1	0.0	0.0	0.0	0.0	0.8	0.6	0.0	0.0
24:1(n-7)c	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0
Sum Monounsaturates	**31.0**	**35.2**	**24.0**	**15.5**	**29.8**	**26.9**	**50.3**	**48.6**	**38.2**	**16.5**
18:2(n-6)	2.1	1.3	0.7	0.6	0.6	1.2	1.6	1.8	2.2	1.6
18:3(n-3)	0.0	0.2	0.0	0.0	0.5	0.5	0.0	0.0	0.0	0.7
18:4(n-3)	2.2	0.2	0.0	0.0	0.1	0.2	0.1	0.1	0.0	0.7
18:3(n-6)	0.0	0.0	1.5	3.2	3.1	2.4	0.0	0.0	0.0	0.0
20:2(n-6)	0.1	0.0	0.0	0.0	0.0	0.0	0.8	1.2	0.8	0.2
20:3(n-6)	0.1	0.0	0.2	0.3	0.4	0.3	0.2	0.0	0.0	0.0
20:4(n-3)	0.2	0.0	0.6	0.7	0.7	0.2	0.4	0.4	0.4	0.3
20:4(n-6)	0.0	0.7	0.0	0.0	0.0	0.0	0.6	0.7	1.2	0.0
20:5(n-3)	17.8	5.1	23.4	31.6	21.9	24.5	6.8	6.7	10.9	20.2
22:5(n-3)	0.4	0.4	0.5	0.6	0.6	0.0	1.1	0.9	0.8	0.4
22:6(n-3)	9.5	8.5	15.2	28.3	13.2	12.6	16.6	15.8	18.1	15.0
Sum PUFA	**32.0**	**16.4**	**42.1**	**65.3**	**41.1**	**41.9**	**28.3**	**27.7**	**34.4**	**39.5**
Other	0.4	0.0	0.0	0.0	0.7	1.1	0.2	0.3	0.0	0.0
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Antarctic Science 9 (3): 258–265 (1997)

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

CHARLES F. PHLEGER[1], PETER D. NICHOLS[2,3] and PATTI VIRTUE[2,3]

[1]*Department of Biology, San Diego State University, San Diego, CA 92182, USA*
[2]*CSIRO, Division of Marine Research, Hobart, Tasmania 7001, Australia*
[3]*Antarctic CRC, Hobart, Tasmania 7001 Australia*

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

Received 16 January 1997, accepted 25 April 1997

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

Introduction

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

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

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

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

Materials and methods

Sample description

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

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

Lipid extraction

Samples were quantitatively extracted using a modified Bligh & Dyer (1959) one-phase methanol: chloroform: water extraction (2:1:0.8 v/v/v); samples were extracted overnight and the phases were separated the following day by the addition of chloroform and water (final solvent ratio, 1:1:0.9 v/v/v methanol: chloroform: water). The total solvent extract (TSE) was concentrated (i.e. solvents removed in vacuo) using rotary evaporation at 30°C. All samples were made up to a known volume in chloroform and stored at -20°C. Samples were stored for up to three days; lipid analysis was conducted immediately.

Lipid classes

An aliquot of the TSE was analysed using an Iatroscan MK V TH10 TLC-FID analyser to determine the abundances of individual lipid classes (Volkman & Nichols 1991). Each sample of the same fish was applied in duplicate or triplicate to silica gel SIII chromarods (5 μm particle size) using 1 μl disposable micropipettes. Chromarods were developed in a glass tank lined with pre-extracted filter paper. The solvent system was hexane-diethyl ether-acetic acid (60:17:0.2 v/v), a mobile phase resolving non-polar compounds such as wax esters, sterol esters, and triacylglycerols, free fatty acids and sterols. A second non-polar solvent system of hexane-diethyl ether (96:4 v/v) was also used for all samples to resolve hydrocarbons, wax esters, triacylglycerols and diacylglyceryl ethers. After development, the chromarods were oven dried and analysed immediately to minimize adsorption of atmospheric contaminants. The flame ionization detector (FID) was calibrated for each compound class (phosphatidylcholine, cholesterol, cholesteryl oleate, oleic acid, squalene, triolein and diacylglyceryl ether, the latter purified from shark liver oil; 0.1–10 μg range). Peaks were quantified on an IBM compatible computer using DAPA software (Kalamunda, Western Australia). Iatroscan results are generally reproducible to ±10% (Volkman & Nichols 1991).

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

Fatty acids and fatty alcohols

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

Gas chromatographic (GC) analyses were performed with a Hewlett Packard 5890A GC equipped with an HP-1 cross-linked methyl silicone fused silica capillary column (50 m x 0.32 mm i.d.), an FID, a split/splitless injector and an HP 7673A auto sampler. Hydrogen was the carrier gas. Following addition of a methyl tricosanoate internal standard, samples were injected in splitless mode at an oven temperature of 50°C. After 1 min, the oven temperature was raised to 150°C at 30C° min^{-1}, then to 250°C at 2C° min^{-1} and finally to 300°C at 5C° min^{-1}. Peaks were quantified with DAPA Scientific Software. Individual components were identified using GC-MS data and by comparing retention times with those

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

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

Determination of double bond configuration in fatty acids and fatty alcohols

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

Results

Lipid content and composition

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

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

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

Fatty acid composition

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

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

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

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

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

Fatty alcohol composition

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

Discussion

Total lipid and lipid classes

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

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

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

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

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

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

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

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

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

Buoyancy implications

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

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

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

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

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

Oily bones

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

Fatty acids and alcohols

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

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

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

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

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

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

Food chain and fisheries implications

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

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

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

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

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

Acknowledgements

This work was supported in part by funding from FRDC. We thank J. Martin and R. Hewitt for the AMLR Expedition to the Elephant Island study area and the Australian National Antarctic Research Expedition for collection of samples in East Antarctica. V. Siegal, D. Outram, and V. Loeb gave invaluable help in collection and identification of samples. C. Phleger thanks the Department of Biology, San Diego State University for sabbatical leave time to work on the research. Ben Mooney gave technical assistance and Danny Holdsworth managed the CSIRO GC-MS facility. We thank Dr N.J. Russell and Dr D.W.H. Walton, and two anonymous journal referees for valuable comments on the manuscript.

References

Ackman, R. 1992. Fatty acids in fish and shellfish. *In* Chow, C.K., *ed. Fatty acids in foods and their health implications*. New York: Marcel Dekker, 169-183.

Adams, N.J. & Brown, C.R. 1989. Dietary differentiation and trophic relationships in the sub-Antarctic penguin community at Marion Island. *Marine Ecology Progress Series*, **57**, 249-258.

Adams, N.J., Moloney, C. & Navarro, R. 1993. Estimated food consumption by penguins at the Prince Edward Islands. *Antarctic Science*, **5**, 245-252.

Bakes, M.J., Elliot, N.G., Green, J.G. & Nichols, P.D. 1995. Variation in lipid composition of some deep sea fish (Teleostei: Oreosomatidae and Trachichthyidae). *Comparative Biochemistry and Physiology*, **111B**, 633-642.

Bligh, E.G. & Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, **37**, 911-917.

Bremner, A., Volkman, J.K., Krasnicki, T. & Gibson, B. 1989. The good oil, 1. Nutritionally important oils in Tasmanian fish. *Australian Fisheries*, **48**, 28-29.

Butler, J.K. & Pearcy, W.G. 1972. Swimbladder morphology and specific gravity of myctophids off Oregon. *Journal of the Fisheries Research Board of Canada*, **29**, 1145-1150.

CCAMLR. 1991. Report of the tenth meeting of the Scientific Committee. Hobart, Tasmania, 21-25th October, 427 pp.

Cherel, Y., Verdon, C. & Ridoux, V. 1993. Seasonal importance of oceanic myctophids in King penguin diet at Crozet Islands. *Polar Biology*, **13**, 355-357.

Childress, J.J. & Nygaard, M.H. 1973. The chemical composition of midwater fishes as a function of depth of occurrence off southern California. *Deep-Sea Research*, **20**, 1093-1109.

Cruickshank, R.A. 1983. Lanternfish ecology in the Benguela Current system. Fifth National Oceanographic Symposium, 24-28 January 1983. Rhodes University, Grahamstown. Abstracts. pp. B51.

Daneri, G.A. & Coria, N.R. 1993. Fish prey of Antarctic fur seals, *Arctocephalus gazella*, during the summer-autumn period at Laurie Island, South Orkney Islands. *Polar Biology*, **13**, 287-289.

Donnelly, J., Torres, J.J., Hopkins, T.L. & Lancraft, T.M. 1990. Proximate composition of Antarctic mesopelagic fishes. *Marine Biology*, **106**, 13-23.

Dunkelblum, E., Tan, S.H. & Silk, R.J. 1985. Double-bond location in monounsaturated fatty acids by dimethyl disulfide derivitization and mass spectrometry. *Journal of Chemical Ecology*, **11**, 265-277.

Elliot, N., Skerrat, J. & Nichols, P. 1990. Orange roughy oil proves its worth. *Australian Fisheries*, **48**, 32-33.

English, R. & Lewis, J. 1991. *Nutritional value of Australian foods*. Canberra: Australian Government Publishing Service, 62 pp

GRIGOR, M.R., SUTHERLAND, W.H. & PHLEGER, C.F. 1990. Wax ester metabolism in the orange roughy *Hoplostethus atlanticus* (Beryciformes: Trachichthyidae). *Marine Biology*, **105**, 223-227.

HAGEN W. 1988. On the significance of lipids in Antarctic zooplankton. *Berichte zur Polarforschung*, 49, 1-117.

HEWITT, R.P. & DEMER, D.A. 1991. Krill abundance. *Nature*, **26**, 353-310.

HODDELL, R. 1996. *Pelagic fish and fish larval distribution in Eastern Antarctic waters (CCAMLR area 58.4.1).* Honours thesis, University of Tasmania, 180 pp. [Unpublished].

HOPKINS, T.C., AINLEY, D.G., TORRES, J.J. & LANCRAFT, T.M. 1993. Trophic structure in open waters of the marginal ice zone in the Scotia-Weddell confluence region during spring (1983). *Polar Biology*, **13**, 389-397.

JAMES, K.A.C., BODY, D.R. & SMITH, W.C. 1986. A nutritional evaluation of orange roughy (*Hoplostethus atlanticus*) using growing pigs. *New Zealand Journal of Technology*, **2**, 219-223.

LEE, R.F. & HIROTA, J. 1973. Wax esters in tropical zooplankton and nekton and the geographical distribution of wax esters in marine copepods. *Limnology and Oceanography*, **18**, 227-239.

LEE, R.F. & PATTON, J.S. 1989. Alcohol and waxes. *In* ACKMAN, R.G., *ed. Marine biogenic lipids, fats, and oils.* Boca Raton, Florida: CRC Press, 73-102.

LUBIMOVA, T.G., SHUST, K.V., TROJANOUSKY, F.M. & SEMENOV, A.B. 1983. On the ecology of mass species of Myctophidae in the Atlantic sector of the Antarctic. *In* SOVIET COMMITTEE OF ANTARCTIC RESEARCH. *The Antarctic*. The Committee Report, **22**, 99-106.

MARTIN, J. 1996. *AMLR 1995/96 Field Season Report LJ-96*. 15. La Jolla, CA: Southwest Fisheries Science Center, Antarctic Ecosystem Research Group, 112 pp.

MENDEZ, E., JACHMANIAN, I. & GROMPONE, M.A. 1993. Lipid distribution in blackbelly rosefish (*Helicolenus doctylopterus lahillei*). *Comparative Biochemistry and Physiology*, **105B**, 193-198.

NEIGHBORS, M.A. 1988. Triacylglycerols and wax esters in the lipids of deep midwater teleost fishes of the Southern California Bight. *Marine Biology*, **98**, 15-22.

NEVENZEL, J.C. 1970. Occurrence, function and biosynthesis of wax esters in marine organisms. *Lipids*, **5**, 308-319.

NEVENZEL, J.C., RODEGKER, W. & MEAD, J.F. 1965. The lipids of *Ruvettus pretiosus* muscle and liver. *Biochemistry*, **4**, 1589-1594.

NEVENZEL, J.C., RODEGKER, W., MEAD, J.F. & GORDON, M.S. 1966. Lipids of the living coelacanth, *Latimeria chalumnae*. *Science*, **152**, 1753-1755.

NEVENZEL, J.C., RODEGKER, W., ROBINSON, J.S. & KAYAMA, M. 1969. The lipids of some lantern fishes (family Myctophidae). *Comparative Biochemistry and Physiology*, **31**, 25-36.

NICHOLS, P.D., NICHOLS, D.S. & BAKES, M.J. 1994. Marine oil products in Australia. *Inform*, **5**, 254-261.

NICHOLS, P.D., VIRTUE, P. & ELLIOTT, N. 1996. *Nutritional value of Australian seafood: oil, fatty acid and cholesterol composition.* Australian Marine Science Association, National Meeting, July. Hobart, Tasmania, 67 pp.

NICHOLS, P.D., GUCKERT, J.B. & WHITE, D.C. 1986. Determination of monounsaturated fatty acid double-bond position and geometry for microbial monocultures and complex consortia by capillary GC-MS of their dimethyl disulfide adducts. *Journal of Microbial Methods*, **5**, 49-55.

NICHOLS, D.S., WILLIAMS, R., DUNSTAN, G.A., NICHOLS, P.D. & VOLKMAN, J.K. 1994. Fatty acid composition of Antarctic and temperate fish of commercial interest. *Comparative Biochemistry and Physiology*, **107B**, 357-363.

PHLEGER, C.F. 1991. Biochemical aspects of buoyancy in fishes. *In* HOCHACHKA, P.W. & MOMMSEN, T.P., *eds. Biochemistry and molecular biology of fishes.* Amsterdam: Elsevier Science Publications, 209-247

PHLEGER, C.F. & GRIGOR, M.R. 1990. Role of wax esters in determining buoyancy in *Hoplostethus atlanticus* (Beryciformes: Trachichthyidae). *Marine Biology*, **105**, 229-233.

PHLEGER, C.F. & WAMBEKE, S. 1995. Bone lipids and fatty acids of Peru Fish. *Comparative Biochemistry and Physiology*, **109**, 145-152.

PICKSTON, L., CZOCHANSKA, Z. & SMITH, J.M. 1982. The nutritional composition of some New Zealand marine fish. *New Zealand Journal of Science*, **25**, 19-26.

PLACE, A.R. 1992. Comparative aspects of lipid digestion and absorption: Physiological correlates of wax ester digestion. *American Journal of Physiology*, **263**, 464-471.

REINHARDT, S.B. & VAN VLEET, E.S. 1986. Lipid composition of twenty-two species of Antarctic midwater zooplankton and fish. *Marine Biology*, **91**, 149-159.

SABOURENKOV, E.N. 1991. *Mesopelagic fish of the southern ocean–summary results of recent Soviet studies.* CCAMLR, Selected Scientific Papers, 1990, 433-457.

SAITO, H. & MURATA, M. 1996. The high content of monoene fatty acids in the lipids of some midwater fishes: family Myctophidae. *Lipids*, **31**, 757-763.

SHOTTON, R. 1996. *Evaluation of commercial mesopelagic fisheries in the Gulf of Oman, Islamic Republic of Iran. Technical Report: The potential viability and management implications of a commercial fishery to exploit mesopelagic resources in the Gulf of Oman.* Rome (Italy): FAO Fi-TCP/IRA/4451 Technical Reports.

SIEGEL, V. & LOEB, V. 1995. Recruitment of Antarctic krill *Euphausia superba* and possible causes for its variability. *Marine Ecology Progress Series*, **123**, 45-56.

SIGURGISLADOTTIR, S. & PALMADOTTIR, H. 1993. Fatty acid composition of thirty five Icelandic fish species. *Journal of the American Oil Chemists Society*, **70**, 1081-1087.

TORRES, J.J., WEIGEL, B.L. & LANCRAFT, T.M. 1984. Metabolism of two Antarctic fish species. *Antarctic Journal of the United States*, **19(5)**, 145-146.

ULBRICHT, T.L.V. & SOUTHGATE, D.A.T. 1991. Coronary heart disease: seven dietary factors. *Lancet*, **338**, 985-992.

VOLKMAN, J.K. & NICHOLS, P.D. 1991. Applications of thin layer chromatography-flame ionization detection to the analysis of lipids and pollutants in marine environmental samples. *Journal of Planar Chromatography*, **4**, 19-26.

WISNER, R.L. 1974. *The taxonomy and distribution of lanternfishes (Family Myctophidae) of the Eastern Pacific ocean.* Navy Ocean Research and Development Activity (NORDA) Report 3. Bay St. Louis, MS, 229 pp.

WOODWARD, D.R., RILEY, M., BUICK, D, NICHOLS, P.D. & NICHOLS, D.S. 1995. Nutritional analysis of the flesh and oil of yolla, the Tasmanian mutton bird *Puffinus tenuirostris*: a useful source of omega-3 fatty acids. *Australian Journal of Nutrition and Dietetic*, **52**, 87-91.

Pergamon
0305-0491(94)00083-2

Comp. Biochem. Physiol. Vol. 110B, No. 1, pp. 267–275, 1995

0305-0491/95 $9.50 + 0.00

Lipid, fatty acid and squalene composition of liver oil from six species of deep-sea sharks collected in southern Australian waters

Michael J. Bakes and Peter D. Nichols

CSIRO Division of Oceanography, GPO Box 1538, Hobart, Tasmania, Australia 7001

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 diol profiles. 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.

Key words: Shark liver oils; SLO; DAGE; Squalene; *Somniosus*; *Centroscymnus*; *Etmopterus*; *Deania*; *Centrophorus*.

Comp. Biochem. Physiol. 110B, 267–275, 1995.

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, but 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 utilization of shark waste should therefore be considered.

Correspondence to: M. J. Bakes, CSIRO Division of Oceanography, GPO Box 1538, Hobart, Tasmania, Australia 7001.
Received 5 November 1993; accepted 9 May 1994.

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 realized. 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*, *Centrophorus scalpratus* 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; the liver was analysed immediately. 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 mechanical maceration of the livers, followed by heating at 50–60°C, separation for 2–4 days and decantation of the oil. At this stage, the separation (and further purification) of shark liver oil represents a new industry in Australia; the technology is therefore evolving and being improved as the industry expands. 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 one and six livers. The catch location and water depth for each species were not recorded. All species, except *S. pacificus*, are caught commercially; *S. pacificus* is a rare species and was analysed for comparative purposes.

As *S. pacificus* is not a commercial species, a commercially produced oil was not available for analysis, and standard laboratory techniques were used to obtain an oil from the liver. A portion of the liver from *S. pacificus* was homogenized using a mortar and pestle, and a known mass was quantitatively extracted using a modified Bligh and Dyer (1959) one-phase method: 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 further stored at −20°C. Samples were not stored for any length of time; analysis was commenced immediately.

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 abun-

dances 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/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 $\pm$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 hr at 80°C. Neutral lipids (e.g. glyceryl ether diols and hydrocarbons) were then extracted into hexane/chloroform (4:1 v/v, 3 × 1.5 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 hr). Products were then extracted into hexane/chloroform (4:1 v/v, 3 × 1.5 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 × 0.32 mm i.d.), an FID and a split/splitless injector. Hydrogen was the carrier gas. Following addition of methyl tricosanoate internal standard, samples were injected in splitless mode at an oven temperature of 50°C. After 1 min, the oven temperature was raised to 150°C at 30°C/min, 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 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 $\pm$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 (of diols) 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 hexaunsaturated 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 and 77%

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 (Brier)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.
For *E. granulosus*, (1) and (2) represent two different samples taken during processing.

and 5 and 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 about 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 m (Buranudeen and Richards-Rajadurai, 1986; Deprez *et al.*, 1990).

Centroscymnus plunketi (plunket shark) liver oil comprised 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 (Craik, 1978; Malins and Barone, 1970; Phleger and Grigor, 1990) 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 *et al.*, 1971). Phleger and Grigor (1990) did show, however, that *Hoplostethus atlanticus*, found at similar depths to these deep-sea sharks, may well use lipid deposits (in this case, wax ester) to control buoyancy. However, the absence of more precise literature on the role of the lipid classes found in these deep-sea sharks, 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

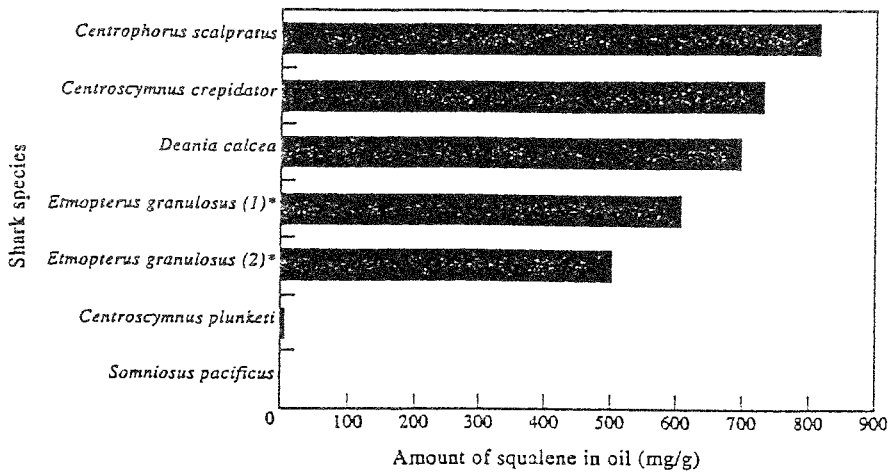


Fig. 1. Absolute amounts of squalene in the liver oil from six species of deep-sea sharks caught in southern Australian waters. For *E. granulosus*, (1) and (2) represent two different samples taken during processing.

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*, *Centrophorus* and *Etmopterus*) in levels of hydrocarbon (squalene), DAGE and TAG. Further studies which examine the effect of depth, geographical distribution and other factors 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*).

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 with, 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 have previously been documented (Summers, 1987). It is our belief that the differences in the oil composition observed reflect real changes in environmental factors, rather than being the result of within-sample variation of individual components. Alternately, small differences in processing conditions may account for the variation observed. However, it was beyond the scope of this study to examine in detail differences in oil yield and composition (epoxide formation, oxidation and other degradation products) with different processing conditions. 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 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 are 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), although, as noted earlier, such a relationship may not necessarily hold for all species.

Glyceryl ether diols (derived from DAGE)

The Iatroscan (TLC–FID) analyser provides a rapid means of detecting DAGE without any sample treatment and derivatization, 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-hexa-decylglyceryl 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 monounsatu-

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

Fatty acid	Percentage composition						
	Somniosus pacificus	*Centroscymnus plunketi*	*Etmopterus granulosus* (2)†	*Etmopterus granulosus* (1)	*Deania calcea*	*Centroscymnus crepidater*	*Centrophorus 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	—	0.4	—
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
*i*17:1	0.3	0.5	0.6	0.6	0.4	0.7	0.5
*i*17:0	0.3	0.3	0.3	0.3	0.3	0.2	0.1
*a*17:0	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
*i*19:0	0.3	0.3	0.3	0.2	0.2	0.2	0.2
*a*19:0	0.4	0.3	0.3	0.3	0.2	0.2	0.2
19:0	0.1	—	—	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*-11)	—	—	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	—	—	0.1	—	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
Sum saturates	12.5	11.5	15.0	16.2	26.1	15.7	25.6
Sum monounsaturates	72.0	83.6	80.0	80.1	64.8	80.2	62.2
Sum polyunsaturates	13.3	2.6	2.5	1.3	7.2	1.41	0.6
Total Fatty Acid (mg/g)	340	575	490	250	175	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 ± %.

†(1) and (2) represent two different samples of liver oil from *E. granulosus* during processing.

— not detected. Lower detection limit for individual compounds was 1 ng (typically 1500 ng of total fatty acids was injected).

rated 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 diacylglyceryl 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, DAGE are not

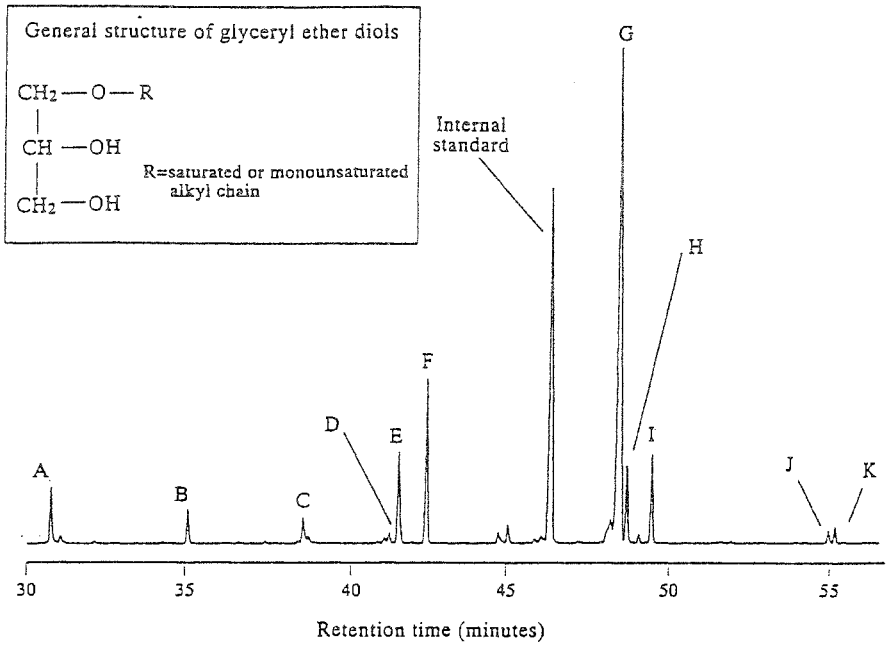


Fig. 2. Capillary gas chromatogram of glyceryl ether diols in neutral lipid fractions of liver oils from six species of deep-sea sharks caught in southern Australian waters. A, unidentified; B, 14:0 diol; C, unidentified; D, unidentified; E, 16:1(n-7)c diol; F, 16:0 diol; G, 18:1(n-9)c diol; H, 18:1(n-7)c;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

widely used commercially, although there has been some suggestions 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.

Acknowledgements—This work was supported in part by funding from FRDC. John Stevens and Stephanie Davenport, CSIRO Fisheries, and Greg Simpson and Chris Strauss, CSIRO Chemicals and Polymers, are thanked for many valuable discussions and on-going support. David Nichols and Graham Green are thanked for laboratory assistance, and John Volkman and Graeme Dunstan are thanked for comments during manuscript preparation. Our colleagues in the Marine Resources and Pollution Program are thanked for assistance with various aspects of the project. BEKU Environmental Products, Scales, Squalus, ITL, Sydney-Wide Seafoods, Trident Seafoods and Tas Crays are thanked for provision of materials and their ongoing interest and support of the project.

References

Batista I. and Nunes M. L. (1992) Characterisation of shark liver oils. *Fish. Res.* **14**, 329–334.

Bligh E. G. and Dyer W. J. (1959) A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37**, 911–917.

Blomstrand R. and Ahrens E. (1959) Absorption of chimyl alcohol in man. *Proc. Soc. exp. Biol. Med.* **100**, 802–805.

Buranudeen F. and Richards-Rajadurai P. N. (1986) Squalene. *Infofish Marketing Digest.* **1**, 42–43

Cowey C. B. and Sargent J. R. (1977) Lipid nutrition in fish. *Comp. Biochem. Physiol.* **57B**, 269–273.

Craik J. C. A. (1978) The lipids of six species of shark. *J. Mar. Biol. Ass.* **58**, 913–921.

Davenport S. R. and Deprez P. P. (1989) Market

opportunities for shark liver oil. *Aust. Fisheries* 48, 8–10.

Deprez P. P. Volkman J. K. and Davenport S. R. (1990) Squalene content and neutral lipid composition of livers from deep-sea sharks caught in Tasmanian waters. *Aust. J. Mar. Freshwater Res.* 41, 375–387.

Dunkelblum E., Tan S. H. and Silk R. J. (1985) Double-bond location in monounsaturated fatty acids by dimethyl disulphide derivatization and mass spectrometry. *J. chem. Ecol.* 11, 265–277

Gopakumar K. and Thankappan T. K. (1986) Squalene, its source, uses and industrial applications. *Seafood Export J.* 17–21.

Hayashi K. and Takagi T. (1981) Distribution of squalene and diacylglyceryl ethers in the different tissues of deep-sea shark, *Dalatias licha*. *Bull. Japn. Soc. Sci. Fish.* 47, 281–288.

Kayama M., Tsuchiya Y. and Nevenzel J. C. (1971) The glyceryl ethers of some shark liver oils. *Bull. Japn. Soc. Sci. Fish.* 37, 111–118.

King K. and Clark M. (1987) Sharks from the upper continental slope—are they of value? *Catch* 14, 3–6.

Malins D. C. and Barone A. (1970) Glyceryl ether metabolism: regulation of buoyancy in dogfish *Squalus acanthias*. *Science* 167, 79–80.

Mangold H. K. (1983) Ether lipids in the diet of humans and animals. In *Ether Lipids. Biochemical and Biomedical Aspects* (Edited by Mangold H. K. and Paltauf F.), pp. 231–237. Academic Press, New York.

Nichols P. D., Guckert J. B. and White D. C. (1986) Determination of monounsaturated fatty acid double-bond position and geometry for microbial monocultures and complex consortia by capillary GC-MS of their dimethyl disulphide adducts. *J. Microbiol Meth.* 5, 49–55

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

Phleger C. F. and Grigor M. R. (1990) Role of wax esters in determining buoyancy in *Hoplostethus atlanticus* (Beryciformes: Tractichthyidae). *Mar. Biol.* 105, 229–233.

Russell N. J. (1990) Cold adaptation of microorganisms. *Phil. Trans. Royal Soc. Lond. B.* 326, 595–611.

Sargent J. R., Gatten R. R. and McIntosh R. (1973) The distribution of neutral lipids in shark tissues. *J. Mar. Biol. Ass.* 53, 649–656.

Summers G. (1987) Squalene—a potential shark by-product. *Catch* 14, 29.

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

RECENT ADVANCES IN MARINE SCIENCE AND TECHNOLOGY '94

Edited by

Orpha Bellwood

and

Howard Choat
Professor, Marine Biology
James Cook University, Australia

and

Narendra Saxena
Professor, Civil Engineering
University of Hawaii, U.S.A.

PACON INTERNATIONAL

and

JAMES COOK UNIVERSITY OF NORTH QUEENSLAND

1994

VALUE-ADDED PRODUCTS FROM AUSTRALIAN SHARK LIVER OILS

Michael Bakes, Peter Nichols[1] and Christopher Strauss[2]

[1] CSIRO Division of Oceanography, GPO Box 1538, Hobart, Tas., 7001, Australia
[2] CSIRO Division of Chemicals and Polymers, Private Bag 10, Rosebank MDC, Clayton, Vic., 3169, Australia.

ABSTRACT

The by-catch and the waste generated by the Australian fishing industry are a resource with considerable economic potential. Scientific research agencies have worked collaboratively with industry to establish new processing techniques to isolate valuable lipids, including hydrocarbons (squalene), from the livers of deep-sea sharks. An increasing global demand for some of these specialised purified chemicals (e.g. machine lubricants, health products, skin moisturisers and other products) and rising costs of waste disposal have encouraged research in this area. The value of a previously wasted resource has now been identified as a commodity worth more than one million dollars (per annum) to Australian suppliers, based on the market demand, price and the current catch rates.

INTRODUCTION

The by-catch generated by deep-sea (600–1200 m) trawling for orange roughy in southern Australian waters includes members of the Oreosomatidae and Macrouridae families (oreos, whip tails and rat tails) and deep-sea dogfish (pers. comm. S. Davenport, CSIRO Div. Fisheries, Hobart). These by-catch species may constitute anything from zero to 100% of a catch, but are usually around 5–10% (Davenport and Deprez, 1989). Some fishers may target sharks when the orange roughy catch is low. Total Allowable Catches (TACs) for orange roughy have been reduced over the past few years and hence the ability to exploit by-catch is increasing in importance. There are currently no quotas for deep-sea sharks from southern waters and other regions, but clearly such a resource should not be wasted.

The species of interest are usually members of the Squalidae family, commonly called dogfishes. They are usually between 50 and 150 cm in length and weigh up to 4 kg (Davenport and Deprez, 1989). The liver may account for up to 20% of the body mass of the shark and the oil can contain 0–90%, — more usually 40–80% — squalene (S. Davenport, unpublished data). They are in fisheries from the Great Australian Bight, around Tasmania, to New South Wales, and in waters around New Zealand (specifically Lord Hoe Rise, Challenger Plateau and Chatham Rise) (Last and Stevens, 1994). Species commonly taken are from the genera *Centrophorus*, *Centroscymnus*, *Etmopterus* and *Deania*. *Centrophorus moluccensis* is also targeted with long-line, as this species is caught in shallower waters (300–500 m).

The current world demand for squalene is at least 1100 to 1200 tonnes per year. The main supplier and user, being Japan, requires 900 tonnes. Australia has recently started exporting squalene derived from shark liver oil and the quantity is expected to increase substantially in the future. Based on a squalene price of US$20–25/kg, the export of purified oil is shortly expected to return more than A$1 million per annum. The market value of squalene does, however, fluctuate. For example, squalene has been worth considerably more during the past decade with a market value over US$40/kg.

In this paper, we discuss the analytical protocol for screening species of deep-sea sharks for squalene and other valuable lipid components. Data are provided on a number of species that are currently targeted

for squalene, and both the commercial incentives and current value for such purified shark liver oils are discussed. This information has not been widely available in Australia.

ANALYTICAL TECHNIQUES

The material analysed was either a portion of frozen raw liver or an aliquot of a crude oil, often prepared by local industry. The processing techniques industry uses to prepare shark liver oil vary, but may involve mechanical maceration of liver, followed by heating at 50–60°C, standing to allow the solids to separate, and finally decanting (or possible centrifuging and filtering).

When we analysed raw livers, we used a modification of the technique developed by Bligh and Dyer (1959) to extract the oil. This oil was then concentrated using rotary evaporation and made up to a known volume in chloroform. Crude oils supplied by industry were diluted with chloroform then directly analysed for lipid composition with a MK V thin-layer chromatograph with flame ionisation detection (TLC-FID, Iatron Laboratories, Japan); Iatroscan results with the MK III analyser are generally reproducible to ±10% (Volkman and Nichols, 1991) and we have recently found the MK V system to be reproducible to ±3-5% (unpublished data).

For the laboratory analyses only, non-saponifiable lipids such as glyceryl ether diols, derived from the diacylglyceryl ethers present in the shark liver oil, and hydrocarbons (predominantly squalene) were isolated together following saponification. Glyceryl ether diols were converted with N,O-bis-(trimethylsilyl)-trifluoroacetamide to their corresponding trimethylsilyl ethers and made up to a known volume with an internal injection standard. Fatty acids (from diacylglyceryl ethers and triacylglycerols present in the crude liver oil) liberated during saponification will not be discussed further in this paper.

Gas chromatographic (GC) analyses of the non-saponifiable lipid fraction were performed with a Hewlett Packard 5890A GC equipped with a 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. After methyl tricosanoate was added as an internal injection standard, samples were injected in splitless mode; the initial oven temperature was 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, Western Australia. Individual components were identified from mass spectral data and from a comparison of retention times with those obtained for authentic and laboratory standards. All GC results are subject to errors 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.

LIPID COMPOSITION OF SHARK LIVERS

The common and scientific names of the shark species analysed are shown in Table 1. The lipid compositions of the liver oils are shown in Figure 1. The major lipids present were diacylglyceryl ethers and triacylglycerols and the main hydrocarbon, squalene. The structures of these compounds are shown in Figure 2.

Table 1. Common and scientific names for shark species examined.

Scientific Name	Common Name
Family Squalidae	
Centrophorus moluccensis Bleeker, 1860	Endeavour dogfish
Centrophorus uyato (Rafinesque, 1810)	Southern dogfish
Centroscymnus crepidater (Bocage & Capello, 1864)	Golden dogfish
Centroscymnus plunketi (Waite, 1910)	Plunkets shark
Deania calcea (Lowe, 1839)	Brier (Platypus) shark
Etmopterus granulosus (Gunther, 1880)	Southern Lantern shark
Family Triakidae	
Galeorhinus galeus (Linnaeus, 1758)	School shark
Mustelus antarcticus Gunther,1870	Gummy shark

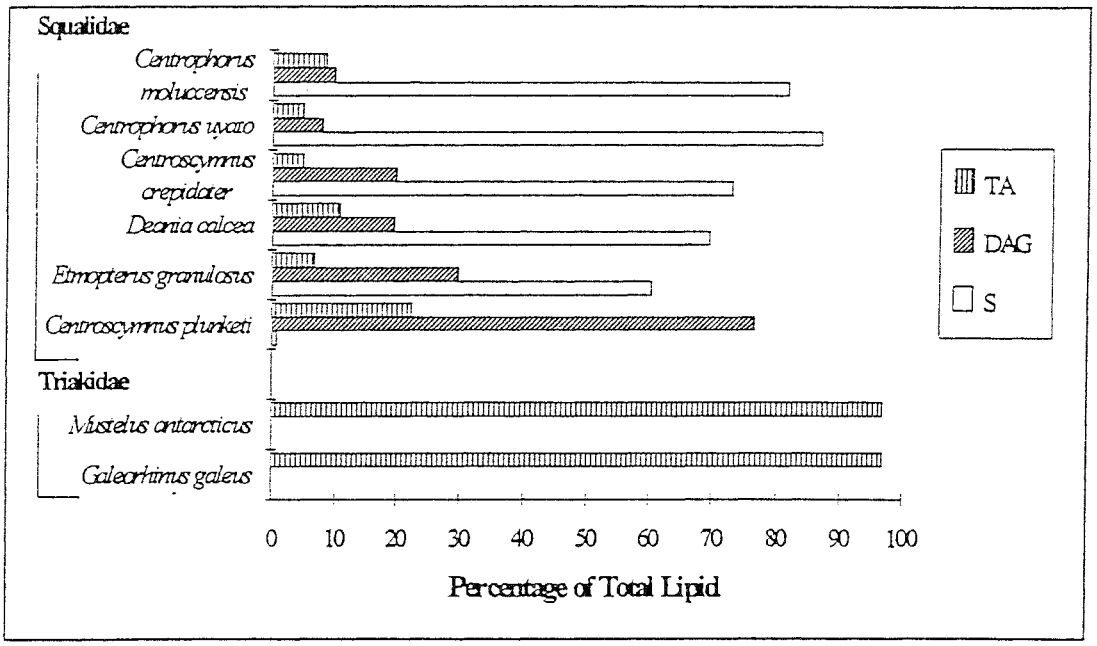


Figure 1. The relative concentrations (as percentage of total lipids) of squalene (SQ), diacylglyceryl ether (DAGE) and triacylglycerols (TAG)in liver oils from sharks caught in Australian waters.

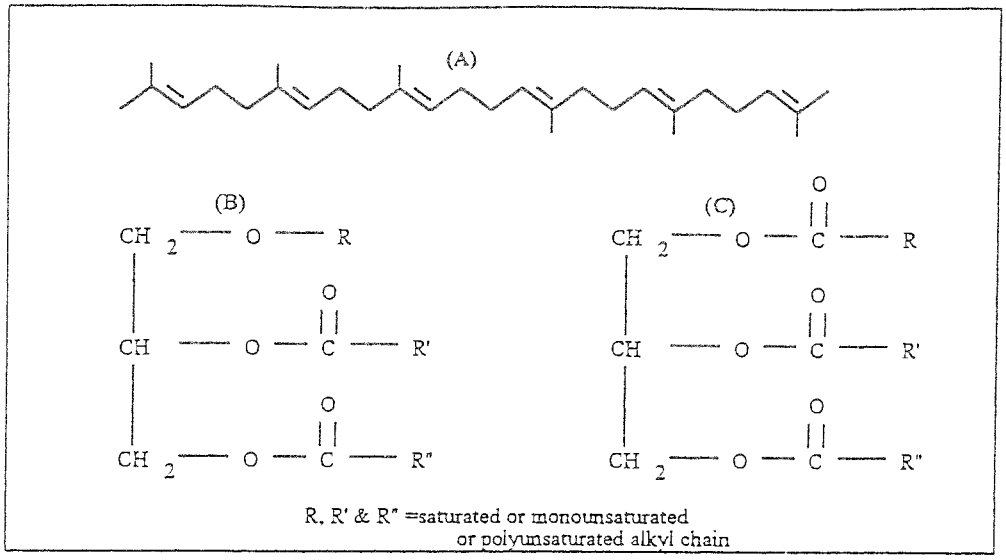


Figure 2. Chemical structures of lipids and hydrocarbons isolated from shark liver oil. (A) squalene, (B) diacylglyceryl ether, (C) triacylglycerol.

Squalene, when present, was generally found at levels between 60 and 90% of the total lipid; sometimes there was none or only a trace (see also Bakes and Nichols 1994; Davenport and Deprez 1989; Deprez *et al.* 1990). The levels of squalene do not appear to be related to the depth of catch or other environmental or geographical factors, but are more probably related to genetic factors. As can be seen from Figure 1, all species tested in the Squalidae family contain more than 50% squalene with the exception of *Centroscymnus plunketi*. The liver oil from members of the Triakidae (*Galeorhinus galeus* and *Mustelus antarcticus*) contained little or no squalene.

Generally, when squalene levels were low in the deep-water dogfishes, levels of diacylglyceryl ethers were higher. A commercial use for diacylglyceryl ethers from shark liver oil has not yet been exploited in Australia. Triacylglycerols are usually present at levels between trace to 50% in the liver oil of deep-sea sharks but higher in the shelf and upper slope water species (e.g. *G. galeus*, 97% triacylglycerols). The commercial value of triacylglycerols is usually based on the chain length and degree of unsaturation of the constituent fatty acids. We do not intend to elaborate on the fatty acid composition of the shark liver lipids here, although we note that the triacylglycerol-rich oils from shark livers may have commercial potential if they contain high levels of the essential polyunsaturated fatty acids [20:5(n-3), eicosapentaenoic acid (EPA); 22:6(n-3), docosahexaenoic acid (DHA)].

COMMERCIAL BACKGROUND

Shark liver oils are not a new resource in Australia. In the late 18th century and early 19th century, oil from shark livers was used as a fuel for lighting. During World War II, the oil was used both as a source of Vitamin A and as a hydraulic fluid in aircraft instrument gauges (Davenport and Deprez, 1989). The use of shark liver oil as a source of Vitamin A ceased after the war and, to the best of our knowledge, has not been re-established, probably because synthetically developed vitamins have been successfully produced (Buranudeen and Richards-Rajadurai, 1986).

Not until the 1980s did Australia begin to apply knowledge of the oils from the livers of deep-sea sharks to value-adding. It took a concerted effort from industry, scientific researchers and other bodies to rebuild interest in the processing of the oils after initial processing trials were not successful. In the 1990s, the timing was appropriate for Australia to add value to this fishery (e.g. Davenport and Deprez 1989). It is

noteworthy that industry was perhaps ahead of Australian researchers in their knowledge of species that were richest in liver oil, although this knowledge was largely anecdotal. It was scientifically verified when species were selected for process development.

The CSIRO Divisions of Oceanography and Chemicals and Polymers have since invented a process to produce high-purity squalene from shark liver oils. CSIRO has worked with small to medium-sized enterprises to transfer both the oil compositional data on Australian deep-sea shark species, and the processing and separation technology. The squalene product from the process is greater than 99% purity, based on GC assay (Figure 3). A second process has also been developed by industry in the past twelve months, so Australia is now well placed to better exploit this once-wasted resource.

The C_{19} saturated isoprenoid hydrocarbon pristane can be present as a minor component in shark liver oils. Pristane (if present, elutes at 15 minutes) and squalene (elutes at 53 minutes) have similar chemical characteristics; however, the difference in the length of the carbon skeleton chain results in significant differences in their physical properties. Distillation can remove these trace quantities of pristane if required.

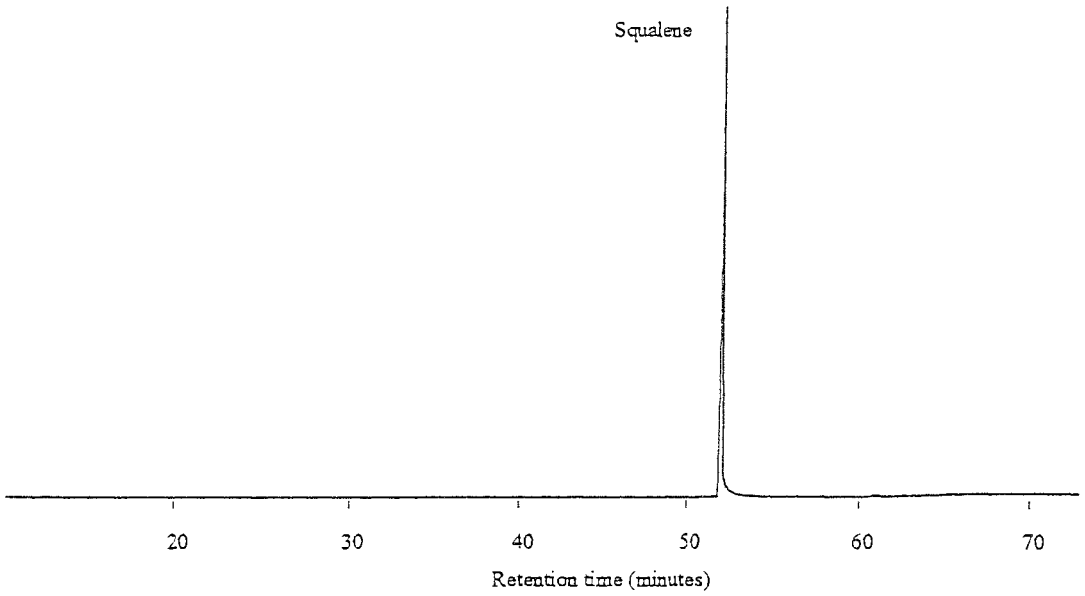


Figure 3. Capillary gas chromatogram of the purified squalene product produced by the CSIRO process.

CURRENT AND FUTURE COMMERCIAL USES AND VALUE

Apart from processing and exporting the purified lipid and hydrocarbon components from shark livers, there is as yet limited product development and usage in Australia. Purified squalene is used overseas in a variety of products, including surface active agents, machine lubricants, rubber chemicals, cosmetics, health products and pharmaceuticals and some food stuffs (Christian 1982; Gopakumar and Thankappan, 1986). Further processing of squalene by hydrogenation to form squalane, the saturated counterpart, results in a number of other uses mainly in the cosmetic industry because of the excellent moisturising properties of the saturated hydrocarbon product. The value of squalane is slightly greater than squalene although the market value has shown considerable variation. Some of the other products currently prepared overseas from squalene and squalane are bath additives, facial cleansers, moisturisers, sun screen and chromatography standards (Christian 1982). A Japanese company has recently begun impregnating cotton clothing (socks, gloves, shirts and shorts) with squalene so that the moisturising effect can be felt during the time the clothing is being worn.

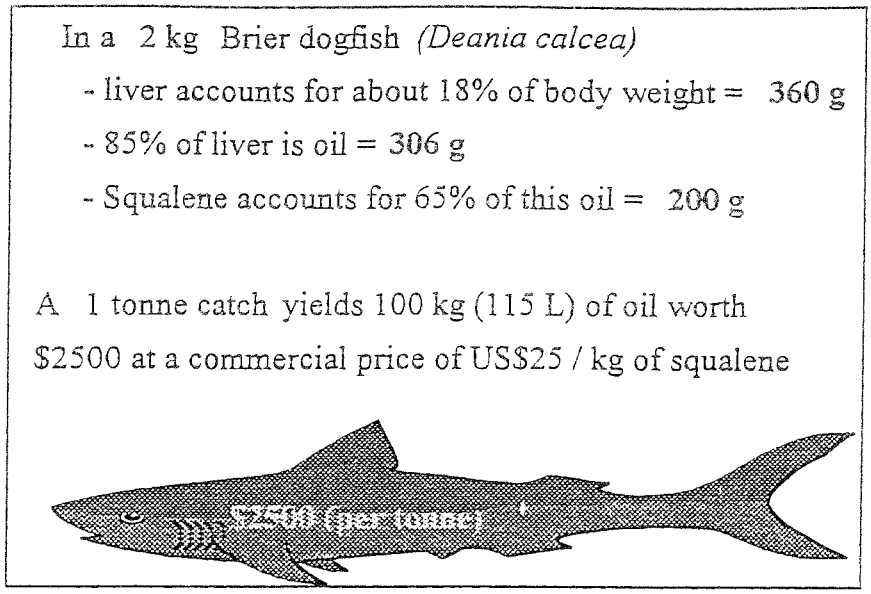


Figure 4. The commercial value of purified squalene obtained from the waste from the deep-sea fishing industry. (a slide designed for the fishing industry presentation)

International factors controlling the prices of purified products are generally outside the scope of the research scientist to monitor. If we consider the above example (Figure 4), using conservative values as indicative only of the potential for value-adding to shark liver oils, the value of a purified product can be ascertained. Based on these estimates, a one tonne catch of Brier dogfish would give a total yield of squalene-rich oil of 100 kg (115 L); its value is over US$2500.

Purified lipids such as diacylglyceryl ethers have been used for a number of purposes. Lion Corporation (Japan) found that ether lipids, particularly monoacylglyceryl ethers, help cure male baldness by encouraging hair growth (Yokoyama *et al.* 1993). Glyceryl ether lipids have also been suggested for use as non-fattening fats, as they have a lower calorific value than triacylglycerols (Mangold and Patlauf 1983) and might be used as low calorie sources of omega-3 fatty acids. Various health products based on diacylglyceryl ethers are available in some countries, including Australia. Products containing diacylglyceryl ethers are claimed to provide concentrated nutritional substances to help prevent or treat health disorders. Although claims have been made that these products have anti-cancer properties, no western medical evidence has been offered to support such claims. Diols formed by hydrolysis of the diacylglyceryl ethers (e.g. batyl and chimyl alcohols) have been also used in cosmetics (Christian 1982), medical applications and for agricultural antidotes (Blomstrand and Ahrens 1959)

The potential of these shark liver-oil constituents needs to be further developed. If additional return can be obtained, the longer-term prospects for the Australian marine oil industry will be considerably enhanced.

In summary, the potential of deep-sea shark liver-oil has (again) been recognised in Australia during the past decade. Now that fractionation and purification techniques are well developed, the opportunity exists to convert resources that have been previously under exploited or wasted into value-added products. The international market fluctuates, so strategies need to be developed to take changes into account. In addition, the "clean and green" nature of the Australian product from pristine waters needs to be appreciated in the market place.

ACKNOWLEDGEMENTS

The authors thank Greg Simpson and Teresa Cablewski (CSIRO Division of Chemicals and Polymers) and Stephanie Davenport and John Stevens (CSIRO Division of Fisheries) for valuable discussions and ongoing contributions. Colleagues in the Division of Oceanography assisted with the project; in particular, David Nichols, Chris Fandry and John Volkman are thanked for their contributions during the early stages of research. We thank Squalus (including for permission to publish certain information), Scales and BEKU Environmental Products Ltd for ongoing support and interest. This work was funded in part by the Fisheries Research and Development Council Grants 91/77 and 94/117.

REFERENCES

Bakes M. J. and Nichols P. D. 1994. Lipid, fatty acid and squalene composition of liver oil from six species of deep-sea sharks collected in southern Australian waters. *Comparative Biochemistry and Physiology*. (in press).

Bligh E. G. and Dyer W. J. 1959. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*. 37: 911–917.

Blomstrand R. and Ahrens E. H. 1959. Absorption of chimyl alcohol in man. *In: Proceedings of the Society of Experimental Biology and Medicine*. 100: 802–805.

Buranudeen F. and Richards-Rajadurai P. N. 1986. Squalene. *Infofish Marketing Digest*. 1: 42–43.

Christian M. 1982. Final report on the safety assessment of squalane and squalene. *Journal of the American College of Toxicology*. 1: 37–56.

Davenport S. and Deprez P. 1989. Market opportunities for shark liver oil. *Australian Fisheries*. 48(11): 8–10.

Deprez P. P., Volkman J. K. and Davenport S. R. 1990. Squalene content and neutral lipid composition of livers from deep-sea sharks caught in Tasmanian waters. *Australian Journal of Marine and Freshwater Research*. 41: 375–387.

Gopakumar K. and Thankappan T. K. 1986. Squalene; its source, uses and industrial applications. *Seafood Export Journal*. (March): 17–21.

Last P. R. and Stevens J. D. 1994. *Sharks and Rays of Australia*. CSIRO Australia. 513 pp + 84 plates.

Mangold H. K. and Patlauf F. 1983. *Ether lipids: Biochemical and Biomedical aspects*. Academic Press, New York.

Volkman J. K. and Nichols P. D. 1991. Applications of thin layer chromatography–flame ionisation detection to the analysis of lipids and pollutants in marine and environmental samples. *Journal of Planar Chromatography*. 4: 19–26.

Yokoyama D., Onodera T., Adachi K. and Oba K. 1993. Effect of glyceryl monopentadecanoate on hair follicles. *Inform*. 4: 480.

Pergamon
0305-0491(95)00024-0

Comp. Biochem. Physiol. Vol. 111B, No. 4, pp. 633–642, 1995

0305-0491/95 $9.50 + 0.00

Variation in lipid composition of some deep-sea fish (Teleostei: Oreosomatidae and Trachichthyidae)

Michael J. Bakes,* Nicholas G. Elliott,† Graham J. Green‡ and Peter D. Nichols*‡

*CSIRO Division of Oceanography, G.P.O. Box 1538, Hobart, Tasmania 7001, Australia;
†CSIRO Division of Fisheries, G.P.O. Box 1538, Hobart, Tasmania 7001, Australia;
‡CRC Antarctic and Southern Ocean Environment, University of Tasmania, G.P.O. Box 252C, Hobart, Tasmania 7001, Australia

The lipid, fatty acid and fatty alcohol compositions were determined for muscle samples from six 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. Similar analyses were also carried out on the muscle and swim bladder of the orange roughy *Hoplostethus atlanticus* from both Australian and North Atlantic waters. Orange roughy is currently a major commercial species in southern Australia and is a new-fishery in the North Atlantic; there are four species of oreo of increasing commercial significance in Australia due to orange roughy quota reductions. It is therefore necessary to determine if the oreo fishing industry is capable of supplementing the current orange roughy requirements with respect to muscle and oil demand. In the oreos, the mean lipid content ranged from 0.5 to 3% of wet weight, with a mixed lipid composition including wax ester, triacylglycerol, sterol and polar lipid. The ratio of the monounsaturated fatty alcohols 22:1 to 20:1 allowed samples from the two geographical regions to be distinguished. Total wax ester in muscle from North Atlantic male orange roughy was much higher than in Australian fish (27 vs. 8.5% wet weight, respectively); females from both locations contained similar amounts of wax ester (4.5 vs. 3.3%, respectively). Selected swim bladders from North Atlantic and Australian orange roughy show similar wax ester content (90 vs. 82%, respectively). 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). Indeed differences exist between oreos from the two locations, but not between orange roughy and this requires further investigation. With respect to the nutritional value, the oreos are more attractive than the orange roughy however lipid levels remain much lower compared with other popular species.

Key words: *Allocyttus* sp.; Fatty acids; Fatty alcohols; *Hoplostethus atlanticus*; *Neocyttus* sp.; *Oreosoma* sp.; *Pseudocyttus* sp.

Comp. Biochem. Physiol. 111B, 633–642, 1995.

Introduction

Fisheries for orange roughy and oreo have recently been established in southeastern Australian waters. Four of the oreos, the black oreo *Allocyttus niger*, the smooth oreo *Pseudocyttus maculatus*, the spiky oreo *Neocyttus rhomboidalis* and the warty oreo *Allocyttus verrucosus*, are currently targeted as commercial species. Current catch rates are about 13,000 tonnes/year for the orange roughy and 3000 tonnes/year for oreo (Nicoll, 1993). The oil

Correspondence to: M. J. Bakes, CSIRO Division of Oceanography, G.P.O. Box 1538, Hobart, Tasmania 7001, Australia. Tel. +61 02 325 222; Fax +61 02 325 123.
Received 15 September 1994; revised 5 January 1995; accepted 10 January 1995.

content of orange roughy can be up to 18% of the total body weight, the majority of which is located in the swim bladder, head, frame and skin (waste products from filleting); the muscle from the orange roughy and the oreo can contain up to 10% of the total lipid (Buisson *et al.*, 1982). Lipid, fatty acid and alcohol compositional data of the muscle from orange roughy (*Hoplostethus atlanticus*) and some of the oreo species have been reported, particularly for species collected in Australian and New Zealand waters (Elliott *et al.*, 1990; Body *et al.*, 1985; Sargent *et al.*, 1983).

The muscle lipids from orange roughy are comprised predominantly of wax esters rich in monounsaturated fatty acids and alcohols. The fatty acid moieties of marine wax esters are usually dominated by polyunsaturated fatty acids (dietary-derived) and the fatty alcohol components are invariably saturated or monounsaturated (synthesized *de novo*) (Sargent *et al.*, 1977). The low levels of polyunsaturated fatty acids and the high levels of monounsaturated fatty acids and alcohols in the wax esters from orange roughy may be a result of several possibilities. Sargent *et al.* (1983) suggested that the monounsaturated alcohols were produced by *de novo* biosynthesis, either by chain elongation and desaturation of shorter chain moieties of dietary origin, or through direct dietary uptake in the form of the acid followed by oxidation or as the alcohol.

Examination of the lipid class, fatty acid and alcohol compositions between the different species of oreo is warranted as the processing waste from these species is now being incorporated into commercial wax ester-rich marine oils. There is increasing evidence that a diet high in $\omega 3$-polyunsaturated fatty acids may help to prevent coronary heart disease and other related diseases (Iacono and Dougherty, 1993; Dyerberg *et al.*, 1978). This feature has resulted in much interest in marine fish and crustaceans as sources of these compounds.

This study presents the lipid, fatty acid and fatty alcohol composition of six species of oreo caught in southern Australian waters, and one in North Atlantic waters. Previous data were only available for three species; the black oreo, smooth oreo and warty oreo (Vlieg and Body 1988; Buisson *et al.*, 1982; Mori *et al.*, 1966). These samples were from different geographical locations (New Zealand and Southern Africa) and consequently, were not representative of Australian fish. We also report a comparison between the lipid, fatty acid and alcohol composition of muscle and swim bladder tissues from orange roughy landed in both Australian and North Atlantic waters and provide some comment on regional dietary relationships.

Materials and Methods

Sample collection

Seven species of oreo were analysed, including the four species caught commercially; *Allocyttus niger* James, Inada and Nakamura (black oreo), *Pseudocyttus maculatus* Gilchrist (smooth oreo), *Neocyttus rhomboidalis* Gilchrist (spiky oreo) and *Allocyttus verrucosus* Gilchrist (warty oreo). The other species analysed were *Oreosoma atlanticum* Cuvier (ox-eyed oreo), *Neocyttus helgae* Holt and Byrne and *Neocyttus* sp. none of which are harvested commercially. All species were caught in Australian waters with the exception of *N. helgae* which was caught in North Atlantic waters (Table 1). Samples of orange roughy (*Hoplostethus atlanticus* Collett) were taken from the major spawning aggregation off eastern Tasmania, and from North Atlantic waters (Table 1).

Except for the North Atlantic samples (*N. helgae* and *H. atlanticus*), all others consisted of whole fish, frozen after capture and transported frozen to the CSIRO Marine Laboratories in Hobart. Muscle samples were taken from similar positions above the lateral line and in front of the dorsal fin (nape region) and the samples stored at −80°C until analysed. The samples from the North Atlantic were taken by the Faroe Islands research vessel *Magnus Heinason*, the oreo *N. helgae* from waters around Faroese (61°39′N, 13°11′W) and the orange roughy from the ICES Fishing Area VIb (between 54°30′N, 60°00′N and 18°00′W, 25°00′W). These tissue samples were dissected at sea, frozen and air-freighted on dry ice to Hobart where they were stored at −80°C prior to analysis.

Lipid extraction

A sample of muscle (0.1–0.5 g) was quantitatively extracted using a modification of the Bligh and Dyer (1959) technique (methanol:chloroform:water; 2:1:0.8 v/v/v). Samples were extracted overnight and phases separated the following day by the addition of chloroform and water so that the final ratio of methanol:chloroform:water was 1:1:0.9 v/v/v. The total solvent extract was concentrated *in vacuo* using rotary evaporation at 30°C. All samples were then transferred to vials, dried under nitrogen at room temperature and made up to a known volume in chloroform. Samples were stored at −20°C.

Lipid composition

Triplicate aliquots of the total solvent extracts were analysed using an Iatroscan MK III TH10 thin layer chromatography–flame ionization detection (TLC–FID) analyser to determine the

Table 1. Location and physical data for oreo dories and orange roughly

Species	Sample code	Sex	Length (SL) (cm)	Catch date	Location
Oreo dory					
Neocyttus rhomboidalis	NR 45	F	32.0	Mar. 92	Great Australian Bight
(spiky oreo)	NR 58	F	18.8	Jun. 92	Challenger plateau, N.Z.
	NR 73	F	18.4	Jun. 92	Great Australian Bight
	NR 116	M	27.7	May 93	West of Tasmania
	NR 117	M	26.3	May 93	West of Tasmania
	NR 122	M	27.3	May 93	West of Tasmania
	NR 123	M	25.2	May 93	West of Tasmania
Oreosoma atlanticum	OA 75	F	16.4	Mar. 92	Great Australian Bight
(ox-eyed oreo)	OA 79	F	14.7	Mar. 92	Great Australian Bight
	OA 84	M	16.6	Mar. 92	Great Australian Bight
Allocyttus verrucosus	AV 527	F	20.2	Apr. 93	South of Tasmania
(warty oreo)	AV 532	F	30.0	Apr. 93	South of Tasmania
	AV 536	F	20.4	Apr. 93	South of Tasmania
	AV 538	F	19.2	Apr. 93	South of Tasmania
	AV 541	F	21.0	Apr. 93	South of Tasmania
Pseudocyttus maculatus	PM 241	M	32.0	May 93	South of Tasmania
(smooth oreo)	PM 242	F	34.2	May 93	South of Tasmania
	PM 243	F	37.0	May 93	South of Tasmania
Allocyttus niger	AN 4	F	27.2	Jan. 92	South of Tasman Rise
(black oreo)	AN 12	F	32.6	Jan. 92	South of Tasman Rise
	AN 14	M	28.6	Jan. 92	South of Tasman Rise
	AN 70	F	32.1	May 93	South of Tasmania
	AN 72	F	31.6	May 93	South of Tasmania
	AN 73	F	33.8	May 93	South of Tasmania
	AN 74	F	29.8	May 93	South of Tasmania
Neocyttus sp.	Na 7	F	21.6	May 93	South of Tasmania
	Na 11	F	16.0	May 93	South of Tasmania
North Atlantic					
Neocyttus helgae	NH 3	M	32.6*	Feb. 93	Faroese
	NH 20	F	27.3*	Feb. 93	Faroese
	NH 25	M	29.5*	Feb. 93	Faroese
Orange roughy					
Tasmanian					
Hoplostethus atlanticus					
	15	M	21	Apr. 89	East of Tasmania
	16	M	38	Apr. 89	East of Tasmania
	1184	F	36.7	Aug. 92	East of Tasmania
	1185	F	39.0	Aug. 92	East of Tasmania
	1187	F	31.1	Aug. 92	East of Tasmania
	1191	F	40.2	Aug. 92	East of Tasmania
North Atlantic					
Hoplostethus atlanticus					
	48	F	62*	Jan. 93	West of Scotland
	49	F	65*	Jan. 93	West of Scotland
	50	F	69*	Jan. 93	West of Scotland
	94	M	61*	Jan. 93	West of Scotland
	95	M	67*	Jan. 93	West of Scotland
	96	M	59*	Jan. 93	West of Scotland
	97	M	62*	Jan. 93	West of Scotland
	98	M	62*	Jan. 93	West of Scotland
	99	M	60*	Jan. 93	West of Scotland
	100	M	66*	Jan. 93	West of Scotland

*Note: all lengths are standard length (SL) unless specified by an asterisk (*) which are total length.

abundances of individual lipid classes (Volkman and Nichols, 1991). The primary 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 hydrocarbons and wax esters (Volkman and Nichols, 1991). The flame ionization detector (FID) was calibrated for each compound class (phosphatidylcholine, cholesterol, cholesteryl ester, oleic acid, squalene, triolein; 0.1–10 μg range). Wax esters have the same R_f as cholesteryl esters under the conditions employed; free sterol determined by TLC–FID was compared with the total sterol content determined by gas chromatography (GC). Using this

Table 2. Lipid class composition and content of oreos from Australian and North Atlantic waters

	Percentage composition Tasmanian						North Atlantic
Lipid class	*Neocyttus rhomboidalis* ($n = 7$) Avg. SD	*Oreosoma atlanticum* ($n = 3$) Avg. SD	*Allocyttus verrucosus* ($n = 5$) Avg. SD	*Pseudocyttus maculatus* ($n = 3$) Avg. SD	*Allocyttus niger* ($n = 7$) Avg. SD	*Neocyttus* sp. ($n = 2$) Avg. SD	*Neocyttus helgae* ($n = 3$) Avg. SD
Wax ester	22 ± 36	9 ± 16	10 ± 15	62 ± 6	52 ± 29	11 ± 13	30 ± 24
Triacylglycerol	6 ± 10	30 ± 33	3 ± 7	7 ± 6	4 ± 10	7 ± 23	25 ± 11
Free fatty acid	10 ± 17	37 ± 38	1 ± 2	0 ± 0	11 ± 17	11 ± 1	19 ± 14
Sterol	5 ± 3	7 ± 2	5 ± 3	0 ± 1	4 ± 3	5 ± 1	3 ± 2
Polar lipid	58 ± 35	16 ± 9	81 ± 21	30 ± 3	30 ± 27	66 ± 20	24 ± 25
Total lipid (mg/g wet)	17 ± 20	5 ± 1	10 ± 8	30 ± 2	29 ± 30	7 ± 2	16 ± 19
Wax ester triacylglycerol ratio	3.9	0.3	2.9	8.6	13.3	1.7	1.2

Avg., average; SD, standard deviation.

comparison, it was found that the wax ester concentration measured by TLC–FID did not contain any sterol esters. Peaks were quantified on an IBM-compatible computer using DAPA software (Kalamunda, Western Australia). Iatroscan results have been previously shown to be reproducible to ±10% (Volkman and Nichols, 1991). Statistics were performed using a one-factor analysis of variance (ANOVA).

Fatty acids and fatty alcohols

An aliquot of the total solvent extract was treated with methanol–hydrochloric acid–chloroform under nitrogen (10:1:1 v/v/v; 80°C, 2 hr) to form fatty acid methyl esters (FAME) and fatty alcohols. Following the addition of water, products were extracted into hexane/chloroform (4:1 v/v, 3 × 1.5 ml), transferred to vials and stored in chloroform. Samples were then dried under nitrogen and treated with *N,O-bis*-(trimethylsilyl)-trifluoroacetamide (BSTFA, 50 μl, 80°C, 1 hr) to convert sterols to their corresponding TMS (trimethylsilyl) ethers. Following silylation, excess BSTFA was removed under nitrogen and the samples made up to a known volume with a solution containing methyl tricosanoate internal standard.

Gas chromatography analyses were performed with a Hewlett-Packard 5890A GC equipped with a HP-1 cross-linked methyl silicone fused silica capillary column (50 m × 0.32 mm i.d.), an FID and a split/splitless injector. Hydrogen was the carrier gas. Samples were injected in splitless mode using an HP 7673 auto injector at an oven temperature of 50°C. After 1 min, the oven 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. Injector and detector temperatures were 290°C and 310°C respectively. Peaks were quantified on an IBM-compatible computer using DAPA software (Kalamunda, Western Australia).

Gas chromatographic–mass spectrometric (GC–MS) analyses were carried out with an 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 an HP 59970C Workstation operated in scan acquisition mode. Typical MSD conditions were: electron multiplier voltage 2200 V, transfer line 310°C, EI energy 70 eV, 0.8 scans/sec and a mass range of 40–650 Da. The GC was fitted with a capillary column similar to that described above. Individual components were identified by comparison of mass spectral data and retention time data with that obtained for authentic and laboratory standards.

Results

Oreos

Lipid content and composition. The lipid composition of muscle from the oreos was dominated by three classes: polar lipid (16–81%), wax ester (9–62%) and triacylglycerol (3–30%) (Table 2); free fatty acid and sterol were minor components, although in some cases, the levels of free fatty acid were quite high (e.g. *O. atlanticum*). In general, *N. rhomboidalis*, *A. verrucosus* and *Neocyttus* sp. had significantly ($P < 0.05$) higher levels of polar lipid (58–81%), while *O. atlanticum* contained significantly ($P < 0.05$) higher levels of both triacylglycerol (30%) and free fatty acid (37%); *P. maculatus* and *A. niger* contained significantly higher levels of wax ester (62 and 52%, respectively). The North Atlantic *N. helgae* contained wax ester, triacylglycerol, polar lipid and free fatty acid in almost equal proportions (30, 25, 24 and 19%,

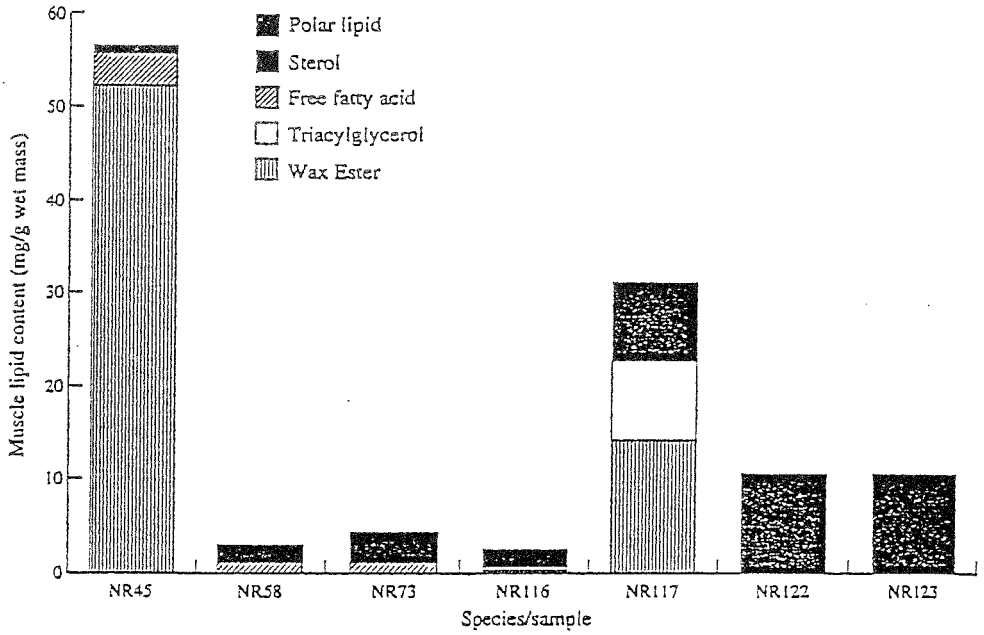


Fig. 1. Comparison of total lipid content and lipid classes in muscle from *Neocyttus rhomboidalis*.

respectively). Mean total lipid ranged from 5 to 30 mg/g (per wet weight of muscle) for the seven species (Table 2); the majority of samples contained less than 20 mg/g of lipid.

Within-species variation was high, as can be seen from the high standard deviations (Table 2). Graphical representation of this variation in lipid content and composition is shown in Fig. 1 for seven samples of *N. rhomboidalis* (NR); wax ester was present in three samples NR45, NR116 and NR117 at between 12 and 93%; triacylglycerol was only present in NR116 and NR117. It is noteworthy that NR116 and NR117 were similar in length, catch location and date, and sex (Table 1). All seven NR samples contained polar lipid (1–97%) and sterol (1–9%), but in different proportions.

Fatty acids. The high degree of variability observed for the lipid content and composition was also observed in the variability of fatty acid composition, both within and between species (Table 3); all oreos except *P. maculatus* had high standard deviations in levels of most fatty acids. Monounsaturated fatty acids were the dominant components in *P. maculatus* (61%) and *A. niger* (52%) and were, in general, significantly higher than in other species. Despite the within-species variation observed, the other five species showed similar levels of all three fatty acid groups (saturated, monounsaturated and polyunsaturated). The major fatty acids were 16:0, 18:0, 18:1(n-9)c, 20:1(n-9)c and the two essential fatty acids eicosapentaenoic acid 20:5(n-3) (EPA, 2–11%) and docosahexaenoic acid 22:6(n-3) (DHA, 5–25%).

Fatty alcohols. The fatty alcohol composition of oreo muscle also showed a high degree of within species variability (Table 3). Monounsaturated alcohols, particularly 20:1(n-9)c and 22:1(n-11)c were the dominant components in all species except *N. rhomboidalis* and *O. atlanticum*; the fatty alcohol composition of these two species was dominated by 16:0 (*N. rhomboidalis* differed significantly from all other species except *O. atlanticum*). Polyunsaturated alcohols were only detected at very low levels and were not present in either *O. atlanticum*, *Neocyttus* sp. or *N. helgae*. The ratio of 22:1(n-11)c to 20:1(n-9)c ranged from 0.2 to 0.9 in Australian caught fish while in *N. helgae* from the North Atlantic, the ratio ranged from 1.8 to 2.2 (Table 3).

Orange roughy

Lipid content and composition. The muscle and swim bladder lipid from orange roughy was comprised almost entirely of wax ester (i.e. 98–100%). The average wax ester content of swim bladders from North Atlantic males (900 mg/g) was similar to that in the swim bladders from Australian males (800 mg/g) and females (820 mg/g) (Table 4; Nichols and Skerratt, unpublished data). For orange roughy from both regions, wax ester was not as concentrated in the muscle (11–390 mg/g) as in the swim bladders. Muscle of North Atlantic male orange roughy contained higher levels of wax

Table 3. Fatty acid (a) and fatty alcohol (b) composition of oreos. All data are presented as average ± standard deviation

Component	Tasmanian						North Atlantic
	Neocyttus rhomboidalis ($n = 5$) Avg. SD	*Oreosoma atlanticum* ($n = 3$) Avg. SD	*Allocyttus verrucosus* ($n = 5$) Avg. SD	*Pseudocyttus maculatus* ($n = 3$) Avg. SD	*Allocyttus niger* ($n = 7$) Avg. SD	*Neocyttus* sp. ($n = 2$) Avg. SD	*Neocyttus helgae* ($n = 3$) Avg. SD
(*a*) *Fatty acids*							
14:0	1.2 ± 0.9	0.2 ± 0.3	0.8 ± 1.2	4.2 ± 1.1	2.1 ± 1.8	1.2 ± 0.5	2.8 ± 0.8
15:0	0.4 ± 0.4	0.1 ± 0.1	0.2 ± 0.3	0.7 ± 0.1	0.3 ± 0.2	0.1	0.2 ± 0.2
16:0	29.5 ± 12.6	26.8 ± 4.4	31.1 ± 7.4	12.2 ± 0.3	19.5 ± 7.2	28.3 ± 13.9	24.4 ± 7.5
17:0	0.7 ± 0.4	0.4 ± 0.2	0.2 ± 0.2	0.7 ± 0.3	0.5 ± 0.3	0.2	0.5 ± 0.5
18:0	8.3 ± 2.4	8.2 ± 2.1	6.4 ± 2.3	2.4 ± 0.2	3.9 ± 1.5	5.8 ± 3.0	5.7 ± 1.3
Saturates	40.1 ± 13.9	35.7 ± 5.8	38.7 ± 8.6	20.1 ± 1.4	26.3 ± 7.1	35.5 ± 17.0	33.5 ± 8.6
16:1(n-7)c	2.1 ± 1.2	1.6 ± 1.0	3.0 ± 1.6	4.5 ± 0.2	3.6 ± 2.0	1.7 ± 0.1	3.3 ± 0.9
18:1(n-9)c	18.4 ± 8.4	16.5 ± 6.8	19.9 ± 4.0	22.8 ± 2.4	21.4 ± 6.3	13.2 ± 1.3	13.9 ± 0.6
18:1(n-7)c	2.6 ± 0.6	2.3 ± 0.3	3.4 ± 0.8	3.5 ± 0.3	3.8 ± 0.8	2.2 ± 0.1	2.3 ± 0.4
20:1(n-9)*c	5.1 ± 4.0	3.7 ± 1.1	5.9 ± 2.0	19.5 ± 0.2	15.4 ± 7.1	14.0 ± 5.9	8.7 ± 1.5
22:1(n-11)c	1.3 ± 1.6	0.4 ± 0.5	0.8 ± 0.8	7.2 ± 0.8	4.4 ± 3.2	4.2 ± 5.1	5.5 ± 0.5
22:1(n-9)c	0.9 ± 0.9	0.2	0.3 ± 0.4	2.3 ± 0.3	1.4 ± 0.7	0.8	0.7 ± 0.6
24:1	1.3 ± 1.0	1.7 ± 0.4	1.1 ± 0.8	1.0 ± 0.2	1.5 ± 0.8	0.8	0.9 ± 0.8
Monounsaturates	31.6 ± 14.5	26.3 ± 9.1	34.3 ± 7.0	60.9 ± 2.8	51.5 ± 16.4	36.9 ± 14.4	35.4 ± 3.5
18:2(n-6)	1.1 ± 0.8	0.7 ± 0.3	0.6 ± 0.6	0.8 ± 0.2	1.2 ± 0.3	1.2 ± 0.3	1.2 ± 0.3
20:4(n-6)	4.3 ± 1.9	3.9 ± 0.8	3.1 ± 1.0	1.9 ± 0.3	2.1 ± 1.2	2.4 ± 0.6	3.3 ± 0.5
20:4(n-3)	0.7 ± 0.5	2.2 ± 3.0	0.7 ± 0.5	1.0 ± 0.3	0.9 ± 0.1	1.6 ± 0.1	0.3 ± 0.3
20:5(n-3)(EPA)	4.5 ± 1.2	6.2 ± 0.8	5.9 ± 1.5	4.1 ± 0.6	5.1 ± 2.9	6.4 ± 0.4	9.7 ± 1.3
22:5(n-3)	1.2 ± 0.9	1.6 ± 0.2	1.9 ± 1.2	1.8 ± 0.4	1.3 ± 0.1	0.8	0.9 ± 0.8
22:6(n-3)(DHA)	16.1 ± 5.3	23.4 ± 1.8	14.8 ± 6.9	9.5 ± 1.9	11.7 ± 6.1	15.6 ± 2.9	15.7 ± 3.7
Polyunsaturates	27.8 ± 8.3	38.0 ± 4.3	26.9 ± 9.7	19.1 ± 3.0	22.3 ± 9.9	27.8 ± 2.8	31.1 ± 5.2
Ratio 22:1/20:1	0.3	0.1	0.1	0.4	0.3	0.3	0.6
(*b*) *Fatty alcohols*							
16:0	43.7 ± 40.0	46.6 ± 48.2	9.1 ± 8.5	7.1 ± 1.3	7.9 ± 3.3	1.7	4.0 ± 3.5
18:0	3.8 ± 6.1	0.5	6.7 ± 8.5	3.6 ± 0.3	4.4 ± 1.1	0.7	4.8 ± 7.3
Saturates	47.5 ± 39.0	47.2 ± 47.5	15.8 ± 16.3	10.7 ± 1.6	12.3 ± 4.4	2.4 ± 3.3	8.8 ± 10.1
18:1(n-9)c	6.9 ± 8.2	9.4 ± 10.7	13.6 ± 13.4	10.7 ± 0.4	15.8 ± 4.7	1.6	1.3 ± 1.1
18:1(n-7)c	1.7 ± 2.0	0.5	10.9 ± 15.7	2.9 ± 0.2	4.2 ± 1.4	0.7	0.9 ± 0.8
20:1(n-9)*c	24.3 ± 20.0	22.9 ± 21.8	44.9 ± 36.1	40.2 ± 1.1	38.0 ± 4.8	57.3 ± 1.8	28.8 ± 4.2
22:1(n-11)c	16.3 ± 14.4	19.3 ± 18.9	12.2 ± 16.5	27.8 ± 0.3	21.8 ± 6.5	34.7 ± 13.2	56.5 ± 10.3
22:1(n-9)c	1.7 ± 2.1	0.7	1.2 ± 1.6	1.8 ± 3.1	3.8 ± 0.9	2.7	2.5 ± 2.3
24:1c	0.9 ± 1.4		0.4 ± 0.6	0.5 ± 0.8	2.2 ± 1.4	0.9	1.1 ± 1.0
Monounsaturates	51.8 ± 38.2	52.8 ± 47.5	83.2 ± 17.1	83.9 ± 5.1	85.8 ± 4.9	97.7 ± 3.3	91.1 ± 10.3
22:6(n-3)	0.7 ± 1.4		0.9 ± 1.3	0.3 ± 0.5	1.4 ± 1.4		
Ratio 22:1/20:1	0.7	0.8	0.3	0.7	0.6	0.6	2.0

*Contains some 20:1.
Avg., average; SD, standard deviation.

ester than their Australian counterparts (270 vs. 85 mg/g) while in the female roughy, the wax ester content of the muscle was similar in specimens from the two regions (45 vs. 33 mg/g) (mean values, Table 4).

Fatty acids. Monounsaturated fatty acids accounted for greater than 90% of the fatty acids in all samples of orange roughy (Table 5). In the North Atlantic orange roughy, the major fatty acids were 22:1(n-11)c, 18:1(n-9)c and 20:1(n-9)c, together comprising between 82 and 84% of the total fatty acids. In contrast, 18:1(n-9)c was the predominant fatty acid in the Australian fish (56% in muscle and 50% in swim bladder). Levels of EPA and DHA were very low in samples from both areas.

Fatty alcohols. There were also major differences in the fatty alcohol composition of the North Atlantic and Australian fish (Table 5). The major fatty alcohol in the muscle of the North Atlantic fish was 22:1(n-11)c, which comprised about 50% of the total fatty alcohols, compared with less than 20% in the muscle of Australian caught fish. The major alcohols in the Australian fish were 20:1(n-9)c (27%) and 16:0 (24%) (Table 5). The composition of fatty alcohols, like the fatty acids, was similar in the swim bladder and muscle of each fish.

Discussion

Oreos

The lipid class data indicated a high degree of within- and between-species variation. Such variation can be attributed in part to a combination of localized (e.g. age, size, sex and reproductive status) and dynamic factors (e.g. seasonal food availability) associated with the fishes' habitat (Stansby, 1976, 1981). This intra-species variation was not associated with the different handling conditions. Proportions of triacylglycerols and wax esters have also been found to be highly variable in other teleost fish (Nevenzel and Menon, 1980); fish living below 250 m used wax ester as their principle energy reserve while epipelagic species used mostly triacylglycerols.

The low lipid content within oreo muscle (range 5–30 mg/g wet) suggests that the muscle in these species is not a site for lipid storage. The amount of lipid in oreos is lower than that in orange roughy (33–270 mg/g wet) and also a number of fish from the U.S.A (6–202 mg/g wet muscle; Kinsella, 1987). As lipid can be deposited throughout the muscle or in specific organs such as the liver, it is not yet possible to comment on whether the low lipid content in the oreos is due to environmental and/or biological factors.

Comparison of the lipid content and composition in the oreos showed that individuals having a high lipid content in their muscle also contained the highest levels of wax ester (e.g. *P. maculatus*, 30 mg/g lipid and 62% wax ester, cf. *N. rhomboidalis* 17 mg/g and 22% wax ester or *A. verrucosus* 10 mg/g and 10% wax ester). Wax esters and triacylglycerols have been described as major metabolic reserves (insulating blankets of fat) and regulators of buoyancy in deep-water species (≈1000 m) such as orange roughy (with approximately 100% wax ester in oil) (Nevenzel, 1970). Wax esters have two distinct advantages over triacylglycerols, being of lower density and being isolated from hormone-controlled mobilisation during energy demand (Grigor *et al.*, 1983; Sargent *et al.*, 1983; Nevenzel, 1970). Given that the oreos and orange roughy occupy similar depths (700–1200 m) and are caught together in trawls, the high degree of lipid variation and the generally low levels of wax ester and triacylglycerols found within the oreos cannot be easily explained without further research. In contrast and as might be expected, absolute levels of polar lipid remained constant across all individuals examined (between 5 and 10 mg/g wet muscle).

Table 4. Wax ester content of orange roughy

Sample code	Flesh [mg/g (wet)]	Swim bladder [mg/g (wet)]
North Atlantic		
Female		
48	51	—
49	60	—
50	24	—
Mean	45	—
Male		
94	390	759
95	272	721
96	121	714
97	314	779
98	352	772
99	345	869
100	117	945
Mean	273	794
Tasmanian		
Male		
15	119	—
16	51	—
Mean	85	—
Female		
1184	40	815
1185	48	822
1187	34	—
1191	11	—
Mean	33	819

In a study of the warty oreo, *A. verrucosus* from southern Africa, Mori *et al.* (1966) reported that the major lipid components were wax ester (76%) and triacylglycerol (17%) (total lipid 4.1%). This differs from the lipid composition found in our study of this species from Australian waters, where polar lipid (45–99%) was dominant with low levels of wax ester (0–34%) and triacylglycerol (0–17%) (total lipid 0.4–2.4%). The higher relative and absolute proportions of wax ester in the southern African fish may be a result of regional specific food chain differences or the difference in the location of the muscle sampled (Phillips, 1991). The warty oreo from South African waters is reported to feed on shrimps, cephalopods and fish (Mel'nihov, 1980). Evidence to date from Australian caught oreos suggests that it and the spiky oreo (*N. rhomboidalis*) have a similar diet to that of warty oreo off South Africa with amphipods, squid, fish and *Pyrosoma* being the major prey (C. Bulman, personal communication).

The correlation of higher levels of monounsaturated fatty acids and higher proportions of wax esters in smooth oreo (*P. maculatus*) and black oreo (*A. niger*) is related to a high dietary predominance of the corresponding alcohols, believed to be zooplanktonic-derived. The principal dietary components for black oreo are amphipods, salps and crustaceans, and for smooth oreo are salps and amphipods (Clark *et al.*, 1989; C. Bulman, personal communication).

The dominance of shorter chain fatty alcohols and fatty acids in oreos (e.g. 16:0, 12–31%) is not unusual as wax esters from several species of

Table 5. Percentage composition of major fatty acids and alcohols in flesh and swim bladders of orange roughy

	North Atlantic (n = 10)				Tasmania (n = 2)			
	Flesh		Swim bladder		Flesh		Swim bladder	
	Avg.	SD	Avg.	SD	Avg.	SD	Avg.	SD
Fatty acids								
14:0	1.5	0.45	1.1	0.3	n.d.	—	1.8	—
16:0	2.1	1.4	1.2	0.2	2.8	0.8	1.9	0.3
Saturates	3.85	—	2.3	—	2.8	—	3.7	—
16:1(n-7)c	6.5	1.6	6.3	0.7	8.4	0.1	10.1	0.9
18:1(n-9)c	28.6	5.1	25.4	3.2	56.2	6.4	49.5	4.9
18:1(n-7)c	4.2	0.7	3.9	0.3	5.7	0.1	4.3	0.2
20:1(n-9)c	22.1	3.9	29.1	2.3	17.2	4.8	21.2	4.5
22:1(n-11)c	30.5	5.3	29.7	3.4	9.1	1.1	8.2	1.6
22:1(n-9)c	2.3	0.5	2.1	0.3	n.d.	—	1.7	
Monounsaturates	93.1	—	96.5	—	96.6	—	95.0	—
20:5(n-3)(EPA)	0.7	0.4	0.6	0.2	0.7	0.1	1.3	0.1
22:6(n-3)(DHA)	1.6	1.8	0.5	0.1	n.d	—	0.5	—
Polyunsaturates	3.15	—	1.1	—	0.7	—	1.8	—
Fatty alcohols								
14:0	0.6	0.2	1	0.2	1.4	0.3	1.9	0.1
16:0	8.6	2	11.6	1.4	24.2	4.2	23.6	1.7
18:0	3.9	0.5	3.7	0.3	9	2.7	7.4	1
Saturates	12.8	—	16.3	—	34.6	—	32.9	—
18:1(n-9)c	4.4	1.3	5.3	0.4	12.9	2.1	13.4	2.5
20:1(n-9)c	20.1	1.9	23.2	1.1	26.5	2.4	29.6	0.1
22:1(n-11)c	53.6	3.8	47.2	1.7	17.3	5.4	15.6	3.7
22:1(n-9)c	4.7	0.9	4.3	0.2	4.7	0.9	4.3	0.6
24:1	4.1	0.3	3.6	0.1	4.1	0.6	4.3	0.1
Monounsaturates	87.3	—	83.6	—	65.5	—	67.2	—

Avg., average; SD, standard deviation; n.d., not detected.

teleost fish were dominated by 16:0 (Sargent *et al.*, 1976). A rare finding in the fatty alcohol composition of the oreos was the presence of the polyunsaturated alcohol 22:6(n-3) in small amounts in all samples except those from *O. atlanticum*, *Neocyttus* sp. and *N. helgae*. This novel polyunsaturated alcohol accounted for 11% of the total alcohols in stomach samples from other *O. atlanticum* samples (unpublished data) suggesting that 22:6(n-3) is most probably of dietary origin. Conversion of the 22:6(n-3) alcohol to the corresponding fatty acid or direct incorporation of 22:6(n-3) alcohol into the muscle as wax ester are the most obvious pathways for the utilisation of the alcohol.

The level of the nutritionally important ω3-fatty acids EPA and DHA in *O. atlanticum* was 29% of the total fatty acids (Fig. 2a), which is considerably greater than levels determined for cod liver oil, jack mackerel oil and some of the commercial ω3-fish oils currently available in Australia (unpublished data). However, on an absolute basis, *O. atlanticum* contained less EPA and DHA (<2 mg/g wet) than many commercial species such as *Platycephalus richardsoni* (tiger flathead, 3.3 mg/g fillet), *Trachurus declivis* (jack mackerel, 15.3 mg/g fillet) and *Latris lineata* (stripped trumpeter, 15.1 mg/g fillet) (D. S. Nicols *et al.*, 1994; P. D. Nichols *et al.*, 1994; Bremner *et al.*, 1989) (Fig. 2b). The other oreo species, whilst having lower relative levels of EPA and DHA (with the exception of *Neocyttus* sp.), had larger amounts of these two essential fatty acids in the muscle (e.g. *A. niger* 4.9, *P. maculatus* 4.1, *N. rhomboidalis* 3.5, *N. helgae* 2.7 mg/g wet; Fig. 2b).

Orange roughy

The wax ester content of muscle from *H. atlanticus* caught in North Atlantic waters was different to that from the same species caught in Australian waters; this finding appeared to be sex dependent. Male North Atlantic fish contained significantly higher amounts of wax ester than their Australian counterparts (273 vs 85 mg/g wet). Females from both regions did not show this trend, but rather contained similar amounts of wax ester. Analyses of the swim bladders indicated similar absolute levels of wax ester (and therefore lipid) in samples from either location.

Studies of the lipids in *H. atlanticus* have been mainly confined to fish caught in New Zealand waters although there has been one study of samples from British waters. Sargent *et al.* (1983) found predominantly wax ester and a

small amount of triacylglycerol in both the swim bladder and muscle from roughy caught in waters west of Britain. The muscle and swim bladder from New Zealand fish also contained large quantities of wax ester (Grigor *et al.*, 1983) suggesting that the fishes from different geographical locations do not vary greatly in their lipid composition. Grigor *et al.* (1983) also reported the wax ester content of the muscle to be 36 mg/g and the swim bladder to be 610 mg/g (wet weight) for New Zealand fish, more similar to Australian fish than North Atlantic fish, as might be expected. Our finding of differences due to sex makes it difficult to further compare Australian and New Zealand orange roughy as biological data for samples analysed by Grigor *et al.* (1983) were not available. As well as the differences in sex, the length (62 cm total length vs. 38 cm standard length) difference between fish from North Atlantic and Australian waters may also contribute to a variation in lipid content.

The fatty acid composition of the swim bladder and muscle of *H. atlanticus* from the two regions differed. The northern hemisphere fish had high levels of 18:1, 20:1 and 22:1, while the southern fish were dominated by 18:1. The high level of 22:1(n-11)c (31%) in muscle of the North Atlantic fish was greater than previously reported for fish caught in Australian (4.7%), New Zealand (7.9%) and British (14.6%) waters (Elliott *et al.*, 1990; Sargent *et al.*, 1983). Similarly, the levels of 18:1(n-9)c in the Australian fish (56% in muscle) are also slightly higher than reported in a previous study of muscle from Australian orange roughy (40–52%; Elliott *et al.*, 1990). These results show that variations in fatty acid composition may also occur within a geographical region and possibly reflect the seasonal and dietary variations in the fishes habitat.

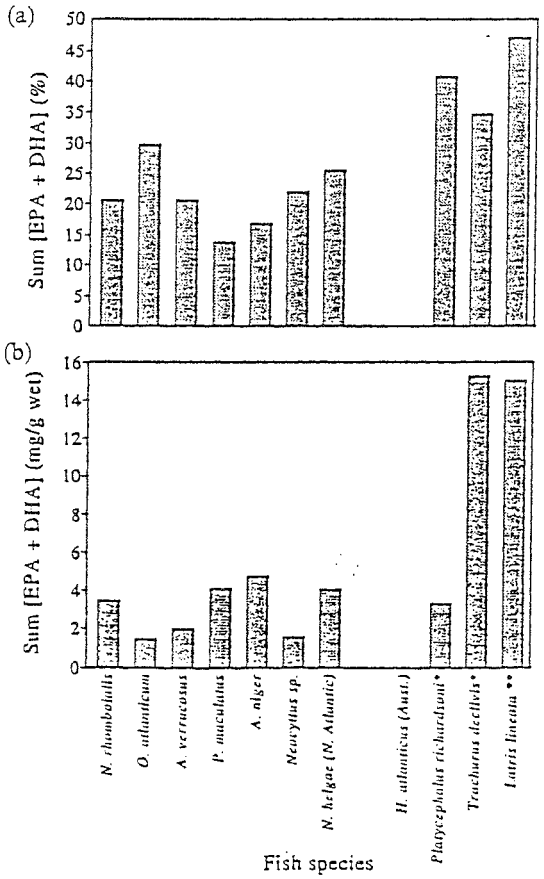


Fig. 2. Comparison between sum (EPA + DHA) (a) as a percentage of the total fatty acids in oreos and other species, and (b) as absolute levels (mg/g) in oreos and other species [*Bremner *et al.* (1989); **D. S. Nichols *et al.* (1994)].

Oreos and orange roughy caught in Australian or North Atlantic waters differed significantly in the ratio of 22:1 to 20:1 fatty alcohols. Australian fish showed a predominance of 20:1(n − 9)c while *N. helgae* and orange roughy from the North Atlantic contained 22:1(n-11)c as the dominant alcohol. The ratio of 22:1 to 20:1 was less than one for Australian fish and two or higher for North Atlantic fish. Our data for orange roughy are similar to the observations by Sargent *et al.* (1983) who recorded a ratio of 22:1 to 20:1 alcohols of 1.3.

The origin and abundance of these alcohols are therefore likely to be due to regional dietary differences rather than factors associated with individual species. The diet of orange roughy from both areas is reported to consist of small fish, squid and crustacea, with the make-up of the latter group differing between regions (Bulman and Koslow, 1993; Rosecchi *et al.*, 1988; Spark, 1993; B. Thomsen, personal communication). In northern waters the diet is mainly comprised of calanoid copepods rich in wax ester and 22:1 fatty alcohol, and in southern waters, mainly euphausids and copepods low in wax esters and 22:1 alcohol.

In summary, from a nutritional view point (in particular ω3-fatty acids) the oreos are relatively rich in DHA with levels generally above 10%. Compared with orange roughy, the muscle from this group of deep water fish is of more value in terms of essential fatty acids; however they are still low in comparison with other more popular species. Unlike the orange roughy, the seven species of oreo examined showed considerable intra-species variation in both lipid content, composition and profiles of fatty acids and alcohols. Individual specimens of each species showed such differences as 0–93% wax ester. These differences, along with differences between male and female specimens in the amount of wax ester in orange roughy and the marked regional differences in the ratio of 22:1 to 20:1

alcohols suggest further investigation is required into lipids of deep water fishes both locally and on a global perspective to better understand the trophodynamics of these species.

Acknowledgements—The authors are grateful to Dr Denis Body (Crop and Food Research Ltd, New Zealand) and our CSIRO colleagues, particularly Malcolm Brown, Cathy Bulman and Graeme Dunstan, for many helpful discussions and suggestions during manuscript preparation. We thank Bjarti Thomsen of the Fisheries Laboratory of the Faroes for obtaining the North Atlantic samples. This study was supported by Fisheries Research and Development Corporation grants 91/77 and 94/115.

References

Bligh E. G. and Dyer W. J. (1959) A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* 37, 911–917.

Body D. R., Johnson C. B. and Shaw G. J. (1985) The monounsaturated acyl and alkyl-moieties of wax esters and their distribution in commercial orange roughy (*Hoplostethus atlanticus*) oil. *Lipids* 20, 680–684.

Bremner A., Volkman J., Krasnicki T. and Gibson B. (1989) The good oil. *Australian Fisheries* 48, 28–29.

Buisson D. H., Body D. R., Dougherty G. J., Eyres L. and Vlieg P. (1982) Oil from deep water fish species as a substitute for sperm whale and jojoba oils. *J. Am. Oil Chemists Soc.* 59, 390–395.

Bulman C. M. and Koslow J. A. (1993) Diet and food consumption of a deep-sea fish, orange roughy *Hoplostethus atlanticus* (Pisces: Trachichthyidae), off south-eastern Australia. *Marine Ecology Progress Series* 82, 115–129.

Clark M. R., King K. J. and McMillan P. J. (1989) The food and feeding relationships of black oreo, *Allocyttus niger*, smooth oreo, *Pseudocyttus maculatus*, and eight other fish species from the continental slope of the south-west Chatham Rise, New Zealand. *J. Fish Biol.* 35, 465–484.

Dyerberg J., Bang H. O., Stoffersen E., Moncada S. and Vane J. R. (1978) Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis? *Lancet* 117–119.

Elliott N., Skerratt J. and Nichols P. D. (1990) Orange roughy oil proves its worth. *Australian Fisheries* 49, 32–33.

Grigor M. R., Thomas C. R., Jones P. D. and Buisson D. H. (1983) Occurrence of wax esters in the tissues of the orange roughy (*Hoplostethus atlanticus*). *Lipids* 18, 585–588.

Iacono J. M. and Dougherty R. M. (1993) Effects of polyunsaturated fats on blood pressure. *Ann. Rev. Nutr.* 13, 243–260.

Kinsella J. E. (1987) Fish and n-3 polyunsaturated fatty acid consumption in the United States: some calculations. In *Seafoods and Fish Oils in Human Health and Disease*, pp. 171–176. Marcel Dekker, New York.

Mel'nikov Yu. S. (1980) Feeding peculiarities of *Allocyttus verrucosus*, family Oreosomatidae. *J. Ichthyology* 20, 99–105.

Mori M., Saito T. and Nakanishi Y. (1966) Occurrence and chemical properties of wax in the muscle of an African fish, *Allocyttus verrucosus*. *Bull. Japan Soc. Sci. Fish.* 32, 668–672.

Nevenzel J. C. (1970) Occurrence, function and biosynthesis of wax esters in marine organisms. *Lipids* 3, 308–319.

Nevenzel J. C. and Menon N. K. (1980) Lipids of midwater marine fish: family Gonostomatidae. *Comp. Biochem. Physiol.* 65B, 351–355.

Nichols D. S., Williams D., 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. (1994) Marine oil products in Australia. *International News on Fats, Oils and Related Materials* 5, 254–261.

Nicoll R. (1993) Grenadier gain softens roughy reduction—AFMA releases 1994 TACs for the SEF. *Australian Fisheries* 52(12), 19–20.

Phillips D. J. H. (1991) The bioaccumulation of trace contaminants in aquatic ecosystems: a review. In *Proceedings of a Bioaccumulation Workshop: Assessment of the Distribution, Impacts and Bioaccumulation of Contaminants in Aquatic Environments* (Edited by Miskiewicz A. G.), pp. 305–322.

Rosecchi E., Tracey D. M. and Webber W. R. (1988) Diet of orange roughy, *Hoplostethus atlanticus* (Pisces: Trachichthyidae) on the Challenger Plateau, New Zealand. *Mar. Biol.* 99, 293–306.

Sargent J. R., Gatten R. R. and McIntosh R. (1977) Wax esters in the marine environment—their occurrence, formation, transformation and ultimate fates. *Mar. Chem.* 5, 573–584.

Sargent J. R., Gatten R. R. and Merrett N. R. (1983) Lipids of *Hoplostethus atlanticus* and *H. mediterraneus* (Beryciformes: Trachichthyidae) from deep water to the west of Britain. *Mar. Biol.* 74, 281–286.

Sargent J. R., Lee R. F. and Nevenzel J. C. (1976) Marine wax esters. In *Chemistry and Biochemistry of Natural Waxes* (Edited by Kolattukudy P.), pp. 50–91 Elsevier, Amsterdam.

Spark A. A. (1993) Wax esters in the marine environment. *Inform* 4(4), 534.

Stansby M. E. (1976) Chemical characteristics of fish caught in the Northeast Pacific Ocean. *Mar. Fish. Rev.* 38, 1–11.

Stansby M. E. (1981) Reliability of fatty acid values purporting to represent composition of oil from different species of fish. *J. Am. Oil Chemists Soc.* 58, 13–16.

Vlieg P. and Body D. R. (1988) Lipid contents and fatty acid composition of some New Zealand freshwater finfish and marine finfish, shellfish, and roes. *N.Z. J. Mar. Freshwater Res.* 22, 151–162.

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 Chromat.* 4, 19–26.

CAROL A. MANCUSO,[A] TOM A. McMEEKIN,[A] DAVID S. NICHOLS[A] MRACI AND PETER D. NICHOLS[A,B] FRACI

The Australian Collection of Antarctic Microorganisms (ACAM): an increasing demand for a new resource

[A] Antarctic CRC, Institute for Antarctic and Southern Ocean Studies, and Department of Agricultural Science, University of Tasmania, Hobart, Tas.
[B] CSIRO Division of Oceanography, Hobart, Tas.

THE AUSTRALIAN Collection of Antarctic Microorganisms* (ACAM) was established in 1986 in the Department of Agricultural Science, University of Tasmania, and has since been enveloped by the Microbial Processes Program of the Antarctic CRC. The five member institutes of the Antarctic CRC are The Australian Antarctic Division, the University of Tasmania, CSIRO Division of Oceanography, The Bureau of Meteorology and The Australian Geological Survey Organisation.

ACAM was set up as a collection for microorganisms isolated from the Antarctic continent as well as from sub-antarctic islands and the Southern Ocean.[1] It is affiliated with the Australian Federation of Culture Collections and the World Federation of Culture Collections. ACAM is one of the few collections in the world dedicated to the collection of Antarctic heterotrophic bacteria.

Since the collection was commenced, many ACAM strains have been isolated from lakes, marine waters, the sea-ice and soils in the Vestfold Hills region of Antarctica near Davis Station. Salinity, oxygen availability, light and temperature all vary dramatically between these water bodies and, on many occasions, can vary greatly with depth within individual lakes. Ace Lake for instance, north-east of the Australian base at Davis Station, is a permanently stratified or meromictic lake. Ice cover, which is present for 9 months of the year or more, prevents wind-induced mixing of colder, saltier oxygen-depleted bottom waters with warmer fresher aerated surface waters. This unusual stratification has resulted in a diverse microbial community whose structure varies with depth through the water column and sediments. Current research involves characterising the microorganisms that inhabit these ecosystems and that are able to tolerate the variety of physical extremes which distinguish the Antarctic environment.

The three key research areas for ACAM to date have been: (1) biodiversity – which microbes are there, (2) biogeochemistry – the role of the microbes in the carbon and other cycles, and (3) biotechnology – the potential commercial exploitation of the microbes.

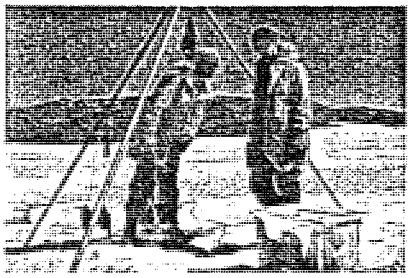
The potential for biotechnological use of Antarctic microorganisms has become increasingly evident from basic studies on their taxonomy and molecular biology. For example, over recent years bacteria have been isolated that (i) produce polyunsaturated omega-3 fatty acids (an important constituent for a healthy diet in humans),[2] (ii) can degrade hydrocarbons including polycyclic aromatics (recalcitrant components of spilled oil), and (iii) can biosynthesise bioactive natural products such as antibiotics or cold/salt-tolerant enzymes.

It is now 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. Other uses also exist. Capsules of fish oils containing high levels of the essential eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3) are marketed aggressively internationally, and have captured a small market in Australia (e.g. Maxepa and related products). More recently omega-3 fatty acids have been incorporated into other food items, for example, they have been enriched in eggs in Australia (through the diet of chickens). The next generation of omega-3 oils contain considerably higher levels of EPA and DHA (approaching 70–80%) and further 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 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% of the essential omega-3 fatty acids EPA and DHA. These two fatty acids are considered to be essential for most species reared by the mariculture

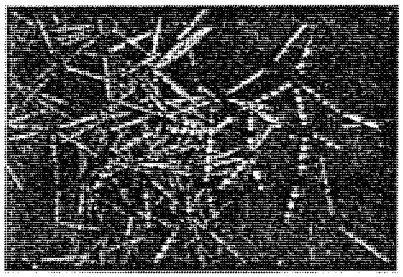
©

* Email address: ACAM@agsci.utas.edu.au



Researchers sampling in Antarctica.

The under-ice environment; the dark green colour is due to the presence of sea-ice algal communities.



A common sea-ice alga *Nitzchia stellata*. The Nile red stain highlights lipid (yellow) stores produced under certain growth conditions against the background of chloroplasts (red).

industry. When the jack mackerel fishery failed in 1989, oils had to be imported into Australia at considerable cost. At that time, data were not available on possible local replacements. By building the database on the oil composition of Australian species and by the possible development of alternative (algal and/or bacterial) sources of materials rich in polyunsaturated fatty acids, we will be much better placed to utilise alternative feed stocks.

The knowledge base and technologies developed with marine oils can be exploited to also isolate valuable oils from algal and bacterial sources. 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 omega-3 polyunsaturated fatty acids (5–20%) and others produce arachidonic acid (20:4ω6). 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 examined the fatty acid composition of approximately 60 Antarctic bacteria. Although a limited number of bacteria have been shown to produce polyunsaturated fatty acids, 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 led 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. In addition to the presence of EPA in many of the Antarctic strains, the level of monounsaturates was considerably higher than observed for many fish species; the level of saturated fatty acids was lower than in fish. Future research in this area will focus on psychrophilic bacteria isolated from the sea-ice environment. In time, chemicals, such as essential fatty acids, made by microalgae and bacteria could be obtained and purified by technology developed for the production of value-added products from fish oils.

ACAM is a continually expanding collection and since its inception has grown from 100 to over 300 strains in early 1995. There is also a growing demand for these unique bacteria from laboratories all over the world. In the last 5 years, ACAM has supplied cultures to researchers in Germany, USA, UK, Denmark, China, Belgium, Egypt, Spain, Japan, Korea and France, as well as many laboratories within Australia.

More recently AMRAD Natural Products Pty. Ltd., an Australian pharmaceutical company, has recognised the biotechnological potential of ACAM as a source of novel bacterial isolates from the Antarctic environment. ACAM is set to expand substantially in the next few years as a result of a recent agreement between the Antarctic CRC and AMRAD. The contract, which provides the Antarctic CRC with its first formal commercial association, has required the clarification of many grey areas of science policy relevant to the Antarctic region. The agreement involves ACAM supplying thousands of microbial isolates from the Antarctic environment which AMRAD will screen for pharmaceutical potential. The funding provided by this agreement will support a large increase in research at the CRC and within ACAM in particular. If the search for human pharmaceuticals proves to be a fruitful one, a resulting royalty stream will be available to fund future Antarctic research.

The search for Antarctic microorganisms that may be commercially exploited has only just begun. Future research should identify novel strains that offer further potential for biotechnology and, at the same time, provide a better understanding of the Antarctic ecosystem.

Acknowledgments

Aspects of the research have been supported by CRC, ASAC, ARC, FRDC and CSIRO–University funding. The authors thank the many people who are part of the ACAM team or who have contributed to ACAM and CSIRO in the past, especially Harry Burton and Peter Franzmann who provided much of the scientific drive during the early development of ACAM.

References

1. C.A. Manusco, K. Sanderson, T. A. McMeekin, P.D. Franzman and H. Burton, "Australian Collection of Antratic Microorganisms: a Catalogue of Strains" (2nd Edn), ANARE Research Note 83, 32 pp. (Australian Government Printing Service, Canberra 1992) Antartic Division DASSET.
2. D. S. Nichols, T. A. McMeekin and P. D. Nichols, *Microbiology*, 1994, 140, 577–584.
3. P. D. Nichols, D. S. Nichols and J. K. Volkman, *Chem. Aust.*, 1993, 60, 336–340.

ELSEVIER

Aquaculture 147 (1996) 115–125

Aquaculture

Enrichment of the rotifer *Brachionus plicatilis* fed an Antarctic bacterium containing polyunsaturated fatty acids

David S. Nichols [a,b,*], Piers Hart [c], Peter D. Nichols [a,d], Tom A. McMeekin [a,b]

[a] *Antarctic CRC, University of Tasmania, GPO Box 252-54, Hobart, Tas. 7001, Australia*
[b] *Department of Agricultural Science, University of Tasmania, GPO Box 252-54, Hobart, Tas. 7001, Australia*
[c] *Department of Aquaculture, University of Tasmania, GPO Box 1214, Launceston, Tas., Australia*
[d] *CSIRO Division of Oceanography, Marine Laboratories, GPO Box 1538, Hobart, Tas. 7001, Australia*

Accepted 17 June 1996

Abstract

The Antarctic bacterium, strain ACAM 456, is known to produce eicosapentaenoic acid (20:5$n-3$, EPA). Following growth in batch culture, suspensions of this bacterium, at initial concentrations of 10^7, 10^8 and 10^9 cells ml^{-1}, were used as foods for three respective cultures of the rotifer *Brachionus plicatilis*. At 6 and 24 h, rotifers were removed, harvested and extracted for analysis of fatty acid composition, which was compared to that of rotifers grown on baker's yeast. Incorporation of EPA, along with bacterial fatty acid markers (i13:0, i15:0 and 14:0), was evidenced at all bacterial food concentrations tested. The highest observed incorporation occurred when rotifers were grown in the medium initially containing 10^9 bacteria ml^{-1}. After 24 h of feeding, the level of EPA reached 9.4% of total fatty acids in the fed rotifers (6.7 ng of EPA rotifer $^{-1}$). ACAM 456, a bacterial strain with the ability to produce EPA, was therefore shown to be a potential alternative enrichment food for the rotifer *Brachionus plicatilis* under feeding conditions that may be applicable to many Australian mariculture operations.

Keywords: Polyunsaturated fatty acid; *Brachionus plicatilis*; Antarctic

* Corresponding author: Tel: +61 0362 202637. Fax: +61 0362 202642. e-mail: D.Nichols@agsci.utas.edu.au

PII S0044-8486(96)01391-9

1. Introduction

The rotifer *Brachionus plicatilis* is widely used as a food for the cultivation of small-mouthed larval fish. However, *B. plicatilis* is incapable of synthesising sufficient polyunsaturated fatty acid (PUFA) components necessary for proper development of larval fish (Lubzens et al., 1985; Rimmer et al., 1994). Rotifers must therefore be enriched in PUFA by feeding with a PUFA-rich food prior to being fed to fish larvae. The nutritive quality of *B. plicatilis* for larval fish development therefore relies on the effectiveness of PUFA incorporation from a PUFA-rich food source. Several techniques have been tested for the enrichment of PUFA in live mariculture feeds such as rotifers, including the use of PUFA-rich microcapsules (Southgate and Lou, 1995) and PUFA-producing bacteria (Watanabe et al., 1992; Yazawa et al., 1992). However, the majority of mariculture operations to date have generally relied upon microalgae as an enrichment feed (Nichols et al., 1989; Watanabe et al., 1992; Lubzens et al., 1995).

The use of microalgae as live enrichment foods incurs considerable expense to many mariculture operations (Lubzens et al., 1995), due to their fastidious growth requirements, e.g. nutrients, light quantity and quality, oxygen and carbon dioxide levels. In contrast, most bacteria are not as fastidious and can often be grown easily on the waste products of agricultural or industrial processes (Nichols et al., 1993).

The utilisation of bacteria as mariculture foods has been an area of international interest for many years (Rieper, 1978; Kiessling and Askbrandt, 1993). However, the perceived lack of PUFA from bacteria was proposed as a major drawback (Phillips, 1984). Now that certain marine bacteria are known to produce high levels of PUFA components (Yazawa et al., 1988; Nichols et al., 1993), the role of microalgae as the sole source of PUFA in natural food chains is also under review (Yazawa et al., 1992). The application of bacterial PUFA production, either from extracts, or via direct insertion into mariculture food chains, is an area of expanding interest (Watanabe et al., 1992; Intriago and Jones, 1993).

Our research has demonstrated that the Antarctic marine environment contains a much higher proportion of PUFA-producing strains than other habitats studied to date (Nichols et al., 1993). Nichols and Russell (1996) have isolated strains with comparable yields of the PUFA, eicosapentaenoic acid (EPA, 20:5$n-3$), to those previously reported. In this study, we investigate the use of an Antarctic PUFA-producing bacterium for the enrichment of the rotifer *B. plicatilis* under feeding conditions applicable to Australian mariculture operations.

2. Materials and methods

2.1. Bacterial strain

Strain ACAM 456 (previously designated strain JS6) was isolated from a sea ice core collected from Prydz Bay, Antarctica (Nichols et al., 1995) and is currently held within the Australian Collection of Antarctic Microorganisms (ACAM, Antarctic CRC, University of Tasmania, Hobart, Tasmania). The bacterium is an unidentified aerobic, Gram-

negative, non-sporulating pleomorphic rod, as described in Nichols et al. (1995). The bacterium is psychrophilic in nature, with an optimal growth temperature of 16.5°C. At the growth temperature utilised in this experiment (10°C), a generation time of 184 min has been determined (Nichols and Russell, 1996).

2.2. Bacterial food

The bacterium was initially grown in 10 ml of Zobell's broth (ZB) (Zobell, 1946) for 2 days at 10°C. This culture was used to inoculate 90 ml of ZB and was incubated at 10°C for 7 days. The 100 ml culture of ACAM 456 was used to inoculate 8.9 l of fresh ZB broth, contained within a sealed 10 l vessel. This was incubated at 10°C for 9 days, with agitation provided by a magnetic stirrer. The culture was harvested by centrifugation using 250 ml vessels (6000 rpm = 3470 g) and the cell pellets were pooled by resuspension in minimal volumes of sterile 0.85% (w/v) saline. Known volumes of the cell resuspension were then taken and serially diluted to obtain absorbance values at 540 nm. From a plot of absorbance versus colony forming units (CFU) ml^{-1} previously prepared for ACAM 456, an estimate of cell concentration per unit volume for the cell suspension was determined (McMeekin et al., 1993). The cell suspension was divided and the resultant fractions were diluted to produce three separate suspensions for rotifer feeding: (i) 1×10^{10} cells in 1 ml; (ii) 1×10^{11} cells in 1 ml and (iii) 1×10^{12} cells in 5 ml.

2.3. Initial rotifer culture

The initial rotifers utilised for the experiment were cultured in 80 l white plastic cylindrical containers with aeration, undergoing a 7-day batch culture cycle. Seawater was maintained at 35‰ salinity and 23°C. The rotifer culture was fed daily with 10–20 l of log-phase microalgal culture (*Isochrysis* sp., Tahitian clone; *Pavlova lutheri* or *Tetraselmis suecica*). Yeast feed (*Saccharomyces cerevisiae*) was also utilised as the microalgae were cleared from the culture vessels, based on visual observations. Assessment of rotifer numbers was carried out by removing three 1 ml samples and placing the contents on petri dishes in small drops prior to counting under a low power microscope. For one day prior to commencement of the experiment, rotifers were fed yeast (*Saccharomyces cerevisiae*) only. Following this period, rotifers were starved for 24 h prior to commencement of the experiment. This procedure took place within the original 80 l culture vessel.

2.4. Rotifer feeding and harvest

Three rotifer cultures were prepared from the initial culture, to which the above bacterial suspensions were added to produce total volumes of 1 l and a rotifer concentration of 200 animals ml^{-1}. A fourth 1 l rotifer culture was prepared at the same concentration, to which 0.2 g of yeast (*Saccharomyces cerevisiae*) was added. Rotifer cultures were maintained at 18°C with constant aeration. After 6 and 24 h, 200 ml fractions of each rotifer culture were removed, the animals collected with a 63 μm mesh

Table 1
Fatty acid composition of foods and rotifer cultures fed yeast or differing concentrations of strain ACAM 456 after 6 and 24 h.

Average percentage composition Sample Code [a] Fatty Acid	Yeast ($n=1$)	ACAM 456 ($n=1$)	0/ROT ($n=2$)	6/YE ($n=2$)	24/YE ($n=2$)	6/107 ($n=2$)	24/107 ($n=2$)	6/108 ($n=2$)	24/108 ($n=2$)	6/109 ($n=2$)	24/109 ($n=2$)
13:0	–	0.5	tr	tr	tr	tr	tr	tr	tr	0.1	0.1
14:0	0.3	13.9	1.7	1.7	1.5	1.7	1.4	2.9	2.2	2.6	4.6
15:0	0.1	2.2	0.5	0.5	0.5	0.5	0.5	0.9	0.8	1.0	1.1
16:0	7.9	23.3	6.2	8.0	7.2	6.7	6.2	8.5	7.9	7.9	7.7
17:0	0.1	0.5	0.4	0.2	0.3	0.3	0.4	0.3	0.4	0.3	0.4
18:0	2.9	2.7	4.9	4.9	4.4	4.8	4.6	4.3	4.4	3.3	3.3
Sum Saturates:	11.4	43.1	13.5	15.3	13.8	14.0	13.0	16.9	15.7	15.2	17.3
i13:0	–	10.8	tr	tr	0.1	tr	0.1	0.5	0.3	3.3	2.3
i15:0	–	4.5	0.7	0.7	0.9	1.0	1.0	1.7	1.3	3.0	3.0
Sum Branched [d]:	–	15.5	1.1	0.7	1.0	1.0	1.3	2.2	1.8	6.7	5.8
14:1$n-8$	–	0.5	0.1	tr	tr	tr	tr	tr	tr	0.1	0.1
14:1$n-7$	0.2	1.1	0.2	tr	0.1	tr	0.2	0.1	0.2	1.0	0.8
15:1$n-8$	–	0.7	0.1	tr	tr	tr	0.2	0.1	0.2	0.6	0.5
16:1$n-9$	–	2.0	1.4	1.1	1.1	1.4	1.3	1.5	1.3	1.9	1.8
16:1$n-7$	17.1	22.8	27.0	29.2	29.3	26.8	26.0	27.6	26.4	33.1	28.9
16:1$n-7$ [b]	–	0.3	0.2	0.3	0.4	tr	0.3	0.1	0.2	0.2	0.4
16:1$n-5$	0.2	tr	0.2	0.1	0.1	tr	0.2	0.1	0.2	0.2	0.2
17:1$n-8$	0.3	0.8	0.7	0.8	0.8	0.7	0.7	0.9	0.9	1.0	0.8
17:1 [c]	–	0.4	0.4	0.3	0.3	0.4	0.5	0.4	0.4	0.4	0.2
18:1$n-9$	44.9	1.9	29.7	31.3	30.1	29.2	27.4	24.1	23.0	16.1	12.7
18:1$n-7$	2.9	2.7	4.4	4.7	6.2	5.7	6.3	5.5	6.1	5.5	6.4
20:1$n-9$	–	–	5.4	4.1	4.2	5.3	5.0	4.4	4.5	2.9	2.9
20:1 [c]	–	–	0.9	0.7	0.8	0.9	0.9	0.8	0.8	0.6	0.7
22:1 [c]	–	–	1.2	0.9	1.0	1.2	1.2	1.0	1.1	0.7	0.7
22:1 [c]	–	–	0.4	0.3	0.3	0.4	0.4	0.5	0.4	0.3	0.3

Sum Monounsat. [e]	65.5	33.2	72.4	73.8	74.8	72.0	70.4	67.2	65.7	64.8	57.6
18:2$n-6$	15.8	0.4	2.2	1.6	1.9	2.2	2.7	1.9	2.8	1.2	2.1
18:3$n-3$	5.9	–	3.4	2.7	2.7	3.4	3.6	2.8	2.9	1.7	1.4
18:4$n-3$	–	tr	0.6	0.5	0.7	0.7	1.0	0.6	1.0	0.3	0.5
20:3$n-6$	–	–	0.6	0.4	0.4	0.5	0.7	0.5	0.6	0.3	0.4
20:2$n-6$	–	–	0.8	0.5	0.6	0.7	0.9	0.6	0.8	0.2	0.3
20:4$n-3$	–	0.4	1.7	1.2	1.2	1.6	1.9	1.5	1.7	1.1	1.5
20:4$n-6$	–	–	1.0	0.8	0.7	1.2	1.4	1.0	1.1	0.3	tr
20:5$n-3$	–	6.8	1.0	1.0	1.0	1.2	1.4	3.3	3.7	7.1	9.4
22:6$n-3$	–	–	tr	tr	0.1	0.1	tr	0.1	0.3	0.4	1.3
Sum PUFA [f]:	21.8	7.6	11.4	8.7	9.4	11.8	13.6	12.1	14.9	12.6	17.1
Other	1.3	0.6	1.6	1.5	0.9	1.2	1.6	1.5	1.9	0.8	2.2
Total:	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
20:5$n-3$ (ng/rotifer)	nd	nd	tr	0.1	0.4	0.1	0.6	0.6	1.6	1.9	6.7
22:6$n-3$ (ng/rotifer)	nd	nd	tr	tr	tr	tr	r	tr	0.1	0.1	0.9

[a] Sample Code Legend (X/Y):
X = 0: Sample time 0 h.
X = 6: Sample time 6 h.
X = 24: Sample time 24 h.
[b] = trans isomer.
Y = ROT: Initial unfed rotifers.
Y = YE: Rotifers fed yeast paste.
Y = 107: Rotifers fed 10^7 bacteria per ml.
Y = 108: Rotifers fed 10^8 bacteria per ml.
Y = 109: Rotifers fed 10^9 bacteria per ml.
[c] Double bond position not determined.
tr = < 0.1% or < 0.1 ng/rotifer, respectively.
nd = not determined.
[d] Includes: (tr–0.3%) i14:0; (tr–0.2%) i16:0; (tr–0.2%) i17.0.
[e] Includes: (tr–0.2%) 15:1$n-6$; all cis geometry unless otherwise stated.
[f] Includes: (tr) amounts of 18:3$n-6$.

screen and washed with filtered seawater (1 l). Suspensions of the washed animals were divided for sample replication and were harvested by filtration onto premuffled (450°C, 24 h) glass-fibre filters (Whatman GF/F) before freezing at −20°C prior to lipid analysis. A sample (1 l) of the initial unfed rotifer culture was also harvested, as described above (see sample 0/ROT, Table 1).

2.5. Lipid extraction and fractionation

Lipids were extracted from bacteria using a modified one-phase (chloroform–methanol–water) Bligh and Dyer extraction (Bligh and Dyer, 1959; White et al., 1979). A portion of the total lipid extract recovered from the lower chloroform phase was used for fatty acid analyses. This portion was transmethylated by reaction for 1 h at 80°C with methanol–hydrochloric acid–chloroform (10:1:1; v/v/v, 3 ml). After the addition of water (1 ml), the resultant fatty acid methyl esters (FAME) were extracted with hexane–chloroform (4:1; v/v, 3 × 2 ml). For gas chromatographic (GC) analysis, samples were diluted with chloroform containing nonadecanoic acid methyl ester (19:0 FAME) as an internal injection standard of known concentration. Peak areas were quantified using chromatography software (DAPA Scientific Software, Kalamunda, Western Australia) operated using an IBM-compatible personal computer. The fatty acid percentages shown in Table 1 represent mean values where duplicate samples have been analysed. Fatty acid concentration data (expressed as ng rotifer^{-1}) were calculated using the initial abundance of rotifers in each culture.

2.6. Analysis of FAME's

GC analyses of the FAME's were performed with a Hewlett Packard 5890 chromatograph equipped with a 50 m × 0.32 mm internal diameter cross-linked methyl silicone (0.17 μm film thickness) fused-silica capillary column and flame ionization detector (FID), as described in Nichols et al. (1995). GC–mass spectrometric (GC–MS) analyses were conducted using a Fisons MD 800 GC–MS system (Barrett et al., 1995). Component identification was by comparison of retention time and MS data with those obtained from authentic and laboratory standards.

3. Results

The fatty acid composition of the yeast- and bacteria-fed rotifers, together with that of the initial unfed rotifers, yeast and bacteria diets, is shown in Table 1. Major fatty acids of the bacterial food, ACAM 456, grown under the conditions described were 16:0, 16:1$n-7$, 14:0, i13:0 and 20:5$n-3$. These components accounted for 77.6% of total fatty acids (TFA). Of particular interest was the level of EPA present in this bacterium (6.8% of TFA under the culture conditions employed). The fatty acid composition of the yeast diet was dominated by 18:1$n-9$ (44.9%), 16:1$n-7$ (17.1%) and 18:2$n-6$ (15.8%). No EPA was detected in the yeast diet, although 18:3$n-3$ was present (5.6%). The fatty acid composition of the initial unfed rotifers (which had not

been fed for 24 h prior to commencement of the experiment) contained 18:1$n-9$, 16:1$n-7$, 16:0 and 20:1$n-9$ as major components, accounting for 68.3% of TFA (Table 1). The depleted nutritional state of the initial unfed rotifers was confirmed by the low level of TFA found in the rotifer sample (5.0 ng of TFA rotifer^{-1}). The level of total PUFA in the initial unfed rotifers was 11.4% of TFA, with the major components being 18:3$n-3$ (3.4%) and 18:2$n-6$ (2.4%). The percentage of EPA was low (1.0% of TFA).

The fatty acid composition of the yeast-fed rotifers at 6 and 24 h is shown in Table 1. Incorporation of the yeast diet by rotifers was evidenced by an increase in the amount of TFA after 6 h (to 14.4 ng rotifer^{-1}) and 24 h (to 37.5 ng rotifer^{-1}) of feeding. However, the fatty acid profile of both the 6 and 24 h yeast-fed samples closely resembled that of the initial unfed rotifer stock culture. 18:3$n-3$ (1.6–1.9%) and 18:2$n-6$ (2.7%) remained the predominant PUFA, with the level of EPA unchanged from that of the initial rotifer sample.

The rotifer culture fed an initial cell concentration of 10^7 bacteria ml^{-1} contained a similar amount of TFA to that of the yeast-fed culture after 6 h feeding (12.0 ng rotifer^{-1}). After 24 h, the level of TFA surpassed that of the yeast-fed samples (44.2 ng rotifer^{-1}). On a comparative basis, the fatty acid composition of rotifers did not differ greatly from the unfed or yeast-fed samples (Table 1).

After 6 h of feeding on 10^8 bacteria ml^{-1}, rotifers contained 17.1 ng of TFA rotifer^{-1}. After 24 h feeding, this value had increased to 43.0 ng rotifer^{-1}. A diet of 10^8 bacteria ml^{-1} led to an increase in both the percentage of bacterial marker fatty acids and EPA in rotifers. After 24 h of feeding, the percentage of EPA reached 3.7% of TFA (Table 1).

Feeding on an initial concentration of 10^9 bacteria ml^{-1} resulted in the highest concentration of TFA found in rotifers at both sampling times (26.0 ng rotifer^{-1} at 6 h; 71.0 ng rotifer^{-1} at 24 h). After both 6 and 24 h of feeding on 10^9 bacteria ml^{-1}, the percentage of bacterial marker fatty acids and EPA increased greatly from the levels present in the initial unfed rotifer culture and the yeast-fed rotifers. The percentages of 18:1$n-9$ and 18:3$n-3$ in rotifer samples fed 10^9 bacteria ml^{-1} decreased after both 6 and 24 h of feeding (Table 1). Comparison of EPA incorporation levels implied a dependence on both the length of feeding and the bacterial food concentration.

Docosahexaenoic acid [22:6$n-3$, DHA] was present as a minor component in both samples of rotifers fed on an initial bacterial concentration of 10^9 cells ml^{-1} (0.4%, 6 h; 1.3%, 24 h). However, rotifers initially fed 10^7 or 10^8 bacteria ml^{-1} contained only trace amounts of DHA, while this component was not detected in either the yeast or bacterial diets (Table 1).

4. Discussion

Watanabe et al. (1992) reported the use of a different EPA-producing bacterium (strain SCRC-6370), thought to be closely related to *Shewanella putrefaciens*, for the nutritive enrichment of *B. plicatilis*. Under the conditions of their study, the highest level of EPA incorporation within rotifers was 7.5% of TFA (2.7 ng rotifer^{-1}) when

rotifers were fed 10^9 bacteria ml^{-1} for 12 h. A close relationship between the level of EPA incorporation and bacterial food concentration was also observed, as indicated in this study (Table 1). EPA incorporation by rotifers from ACAM 456 reached a higher percentage and absolute value than that found by Watanabe et al. (1992) for the bacterium SCRC-6370, however, this was over a longer feeding time. This highlights the need for the individual investigation of differing PUFA-producing bacteria for rotifer enrichment, as has been the case for many microalgal species (Whyte and Nagata, 1990; Jeffrey et al., 1994).

Trophic transfer of bacterial fatty acids was demonstrated by the increased levels of bacterial marker fatty acids (14:0, i13:0 and i15:0) found in rotifers after feeding on ACAM 456. The increasing percentage of bacterial marker fatty acids and EPA found in rotifers when fed increasing concentrations of ACAM 456 implies a direct enrichment mechanism, similar to that observed for the major fatty acid components of microalgal diets (Ben-Amontz et al., 1987; Whyte and Nagata, 1990). Furthermore, the apparent stability of the total percentage of the components (14:0, total branched-chain fatty acids, EPA, $18{:}1n-9$ and $18{:}3n-3$) irrespective of rotifer diet or feeding time (Fig. 1), implies a simple substitution of major fatty acid components of yeast derivation (i.e. $18{:}1n-9$ and $18{:}3n-3$) with those of bacterial diet origin.

The apparent efficiency of EPA incorporation, on a comparative percentage basis, from a food source to the rotifer appears to be variable, and is therefore an issue worthy of consideration when testing new enrichment regimes. Whyte and Nagata (1990) report high levels of incorporation efficiency from *Thalassiosira pseudonana* (which contained

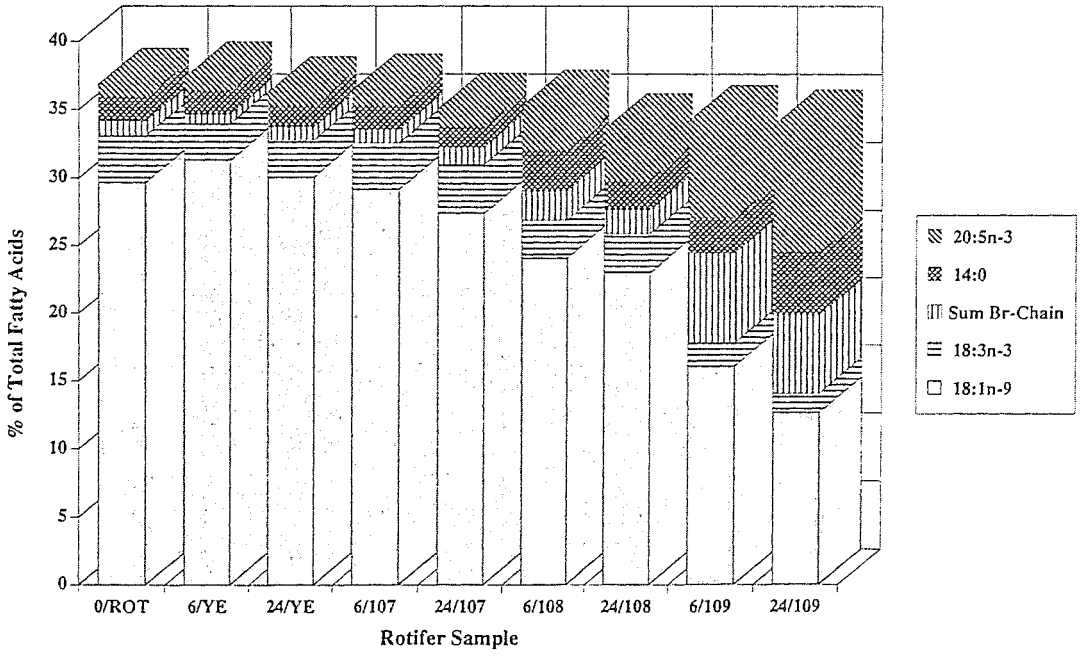


Fig. 1. Changes in the average percentage of bacterial marker fatty acids (14:0, and the sum of branched-chain fatty acids) together with $18{:}1n-9$, $18{:}3n-3$ and $20{:}5n-3$ (EPA) in rotifer samples as a function of diet and feeding time. Sample codes refer to Table 1.

lutheri over a given time period (Volkman et al., 1989; Jeffrey et al., 1994; Nichols and Russell, 1996; unpublished data).

(3) As an extension of this concept, the use of bacteria as a PUFA source may allow the production of co-culture foods; the combined culture of two or more bacterial strains to combine several desired characteristics. For example, the provision of DHA in mariculture diets is an area of expanding interest (Jeffrey et al., 1994). Bacterial strains with the ability to produce DHA have been isolated (Hamamoto et al., 1995). A co-culture of EPA- and DHA-producing bacteria may provide a mariculture food that is rich in both of these essential fatty acids. In addition, the use of probiotics in rotifer cultures is also receiving worldwide attention (Gatesoupe et al., 1989; Gatesoupe, 1994; Bergh, 1995). PUFA-producing bacteria may have further potential as components in a probiotic cocktail.

The present study has demonstrated the effectiveness of an Antarctic PUFA-producing bacterium, ACAM 456, as an enrichment food for the rotifer *B. plicatilis*. The findings encourage the discussion and development of potential bacteria-based enrichment foods by the mariculture industry that are based upon their inherent cultivation and cost advantages.

Acknowledgements

The authors would like to thank Mr. Alex Sobolewshi, who cultured the rotifers and algae used in this experiment. DSN acknowledges the financial support of APA and Antarctic CRC awards. This work was supported in part by ASAC, FRDC (94/115) and ARC. Dr. June Olley and Mr. Tom Lewis are thanked for useful input to the manuscript.

References

Barrett, S.M., Volkman, J.K., Dunstan, G.A. and Leroi, J.M., 1995. Sterols of 14 species of marine diatoms (Bacillariophyta). J. Phycol., 31: 360–369.

Ben-Amontz, A., Fishler, R. and Schneller, A., 1987. Chemical composition of dietary species of marine unicellular algae and rotifers with emphasis on fatty acids. Mar. Biol., 95: 31–36.

Bergh, Ø., 1995. Bacteria associated with early life stages of halibut, *Hippoglossus hippoglossus* L, inhibit growth of a pathogenic *Vibrio* sp. J. Fish Diseases, 18: 31–40.

Bligh, E.G. and Dyer, W.J., 1959. A rapid method for total lipid extraction and purification. Can. J. Biochem. Physiol., 37: 911–917.

Gatesoupe, F.J., 1994. Lactic acid bacteria increase the resistance of turbot larvae, *Scophthalmus marimus*, against pathogenic vibrio. J. Aquat. Living Resour., 7: 277–282.

Gatesoupe, F.J., Arakawa, T. and Watanabe, T., 1989. The effect of bacterial additives on the production rate and dietary value of rotifers as food for Japanese flounder, *Paralichthys olivaceus*. Aquaculture, 83: 39–44.

Hamamoto, T., Takata, N., Kudo, T. and Horikoshi, K., 1995. Characteristic presence of polyunsaturated fatty acids in marine psychrophilic vibrios. FEMS Microbiol. Lett., 129: 51–56.

Intriago, P. and Jones, D.A., 1993. Bacteria as food for *Artemia*. Aquaculture, 113: 115–127.

Iwanami, H., Yamaguchi, T. and Takeuchi, M., 1995. Fatty acid metabolism in bacteria that produce eicosapentaenoic acid isolated from sea urchin *Strongylocentrotus nudus*. Nippon Suissan Gakkaishi, 61: 205–210.

20.5% EPA) and *Tetraselmis suecica* (4.9% EPA) when these microalgae were fed to *B. plicatilis* continuously over a 15 day period (yielding 23.3 and 6.0% EPA of rotifer TFA, respectively). However, in the same experiment, *Isochrysis galbana* (T-iso) (2.2% EPA) yielded a poor efficiency of incorporation into the rotifers (0.8% EPA). Considering that ACAM 456 contained 6.8% EPA, and the level of EPA incorporation achieved within rotifers fed ACAM 456 was 9.4% of TFA (24 h, 10^9 bacteria ml^{-1}), the efficiency of EPA incorporation from ACAM 456 appears to be very good relative to that found in other studies. The EPA present in ACAM 456 is associated with phospholipid species (unpublished data). The observed differences in levels of PUFA incorporation achieved from various enrichment diets may be due to differences in the lipid classes in which the PUFA was present in the food organism.

The level of EPA incorporation using ACAM 456 may also show room for improvement. Nichols and Russell (1996) have reported EPA levels from this bacterium of up to 16% of TFA under laboratory-scale culture conditions. Comparison of the fatty acid composition obtained from the large scale culture used in this study (Table 1) to that obtained by Nichols and Russell (1996) for laboratory-scale cultures, indicates that the decrease in the percentage of EPA was offset by a much higher percentage of saturated fatty acids, particularly 14:0. PUFA biosynthesis appears to rely on an aerobic mechanism, requiring (or preferring) oxygen as a terminal electron acceptor for desaturase enzyme systems (Nichols et al., 1992; Iwanami et al., 1995). ACAM 456 may have suffered oxygen limitation in large culture, resulting in partial inhibition of cell growth and PUFA production. The increased percentage of 14:0 in the large scale culture of ACAM 456 was also detrimental to EPA enrichment, as 14:0 was enriched in a similar manner to EPA in rotifers fed 10^8 or 10^9 bacteria ml^{-1} (Fig. 1). Large scale batch culture of ACAM 456 may require optimisation in terms of EPA production by the bacterium.

The occurrence of DHA as a minor component in rotifers fed 10^8 or 10^9 bacteria ml^{-1} is noteworthy, as this component was absent from both rotifer feeds, and only a minor component (trace to 0.1%) of other rotifer samples. The presence of DHA in rotifer samples that had accumulated elevated levels of EPA is suggestive of chain elongation and desaturation of EPA to DHA by the rotifers (Lubzens et al., 1985).

The major cost, both financially and logistically, incurred in the raising of larval fish in many hatcheries, is the supply of PUFA enrichment diets to rotifers (Jeffrey et al., 1994; Lubzens et al., 1995). The use of bacterial enrichments offers several advantages when compared to the traditional use of microalgae:

(1) The culture of bacteria opposed to microalgae is a simpler operation, in terms of culture conditions and requirements (e.g. light, CO_2, etc.).

(2) Batch cultures of bacteria usually achieve a much higher cell density than those of most microalgal species, and in a much shorter time. For example, in the time required to grow a single batch culture of a microalga, many batch cultures of a bacterium may be produced, yielding a far greater biomass. The greater bacterial biomass produced may offset the higher lipid levels present in most microalgae (Volkman et al., 1989). From a comparison with the microalga, *Pavlova lutheri*, when growth rates, biomass yield and PUFA content are considered, it is estimated that the EPA yield from ACAM 456 would be greater than that obtained from *P.*

lutheri over a given time period (Volkman et al., 1989; Jeffrey et al., 1994; Nichols and Russell, 1996; unpublished data).

(3) As an extension of this concept, the use of bacteria as a PUFA source may allow the production of co-culture foods; the combined culture of two or more bacterial strains to combine several desired characteristics. For example, the provision of DHA in mariculture diets is an area of expanding interest (Jeffrey et al., 1994). Bacterial strains with the ability to produce DHA have been isolated (Hamamoto et al., 1995). A co-culture of EPA- and DHA-producing bacteria may provide a mariculture food that is rich in both of these essential fatty acids. In addition, the use of probiotics in rotifer cultures is also receiving worldwide attention (Gatesoupe et al., 1989; Gatesoupe, 1994; Bergh, 1995). PUFA-producing bacteria may have further potential as components in a probiotic cocktail.

The present study has demonstrated the effectiveness of an Antarctic PUFA-producing bacterium, ACAM 456, as an enrichment food for the rotifer *B. plicatilis*. The findings encourage the discussion and development of potential bacteria-based enrichment foods by the mariculture industry that are based upon their inherent cultivation and cost advantages.

Acknowledgements

The authors would like to thank Mr. Alex Sobolewshi, who cultured the rotifers and algae used in this experiment. DSN acknowledges the financial support of APA and Antarctic CRC awards. This work was supported in part by ASAC, FRDC (94/115) and ARC. Dr. June Olley and Mr. Tom Lewis are thanked for useful input to the manuscript.

References

Barrett, S.M., Volkman, J.K., Dunstan, G.A. and Leroi, J.M., 1995. Sterols of 14 species of marine diatoms (Bacillariophyta). J. Phycol., 31: 360–369.

Ben-Amontz, A., Fishler, R. and Schneller, A., 1987. Chemical composition of dietary species of marine unicellular algae and rotifers with emphasis on fatty acids. Mar. Biol., 95: 31–36.

Bergh, Ø., 1995. Bacteria associated with early life stages of halibut, *Hippoglossus hippoglossus* L, inhibit growth of a pathogenic *Vibrio* sp. J. Fish Diseases, 18: 31–40.

Bligh, E.G. and Dyer, W.J., 1959. A rapid method for total lipid extraction and purification. Can. J. Biochem. Physiol., 37: 911–917.

Gatesoupe, F.J., 1994. Lactic acid bacteria increase the resistance of turbot larvae, *Scophthalmus marimus*, against pathogenic vibrio. J. Aquat. Living Resour., 7: 277–282.

Gatesoupe, F.J., Arakawa, T. and Watanabe, T., 1989. The effect of bacterial additives on the production rate and dietary value of rotifers as food for Japanese flounder, *Paralichthys olivaceus*. Aquaculture, 83: 39–44.

Hamamoto, T., Takata, N., Kudo, T. and Horikoshi, K., 1995. Characteristic presence of polyunsaturated fatty acids in marine psychrophilic vibrios. FEMS Microbiol. Lett., 129: 51–56.

Intriago, P. and Jones, D.A., 1993. Bacteria as food for *Artemia*. Aquaculture, 113: 115–127.

Iwanami, H., Yamaguchi, T. and Takeuchi, M., 1995. Fatty acid metabolism in bacteria that produce eicosapentaenoic acid isolated from sea urchin *Strongylocentrotus nudus*. Nippon Suissan Gakkaishi, 61: 205–210.

Jeffrey, S.W., Brown, M.R. and Garland, C.D., 1994. Microalgae for Mariculture. Final Report to the Fisheries Research and Development Corporation, PO Box 222, Deakin West 2600, Australian Capital Territory, 79 pp.

Kiessling, A. and Askbrandt, S., 1993. Nutritive value of two bacterial strains of single-cell protein for rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 109: 119–130.

Lubzens, E., Marks, A. and Tietz, A., 1985. De novo synthesis of fatty acids in the rotifer *Brachionus plicatilis*. Aquaculture, 47: 27–37.

Lubzens, E., Gibson, O., Zmora, O. and Sukenik, A., 1995. Potential advantages of frozen algae (*Nannochloropsis* sp.) for rotifer (*Brachionus plicatilis*) culture. Aquaculture, 133: 295–309.

McMeekin, T.A., Olley, J.N., Ross, T. and Ratkowsky, D.A., 1993. Predictive Microbiology: Theory and Application. Research Studies Press, Taunton, UK, pp. 71–87.

Nichols, D.S. and Russell, N.J., 1996. Fatty acid adaptation in an Antarctic bacterium — changes in primer utilisation. Microbiology, 142: 747–754.

Nichols, D.S., Nichols, P.D. and McMeekin, T.A., 1992. Anaerobic production of polyunsaturated fatty acids by *Shewanella putrefaciens* strain ACAM 342. FEMS Microbiol. Lett., 98: 117–122.

Nichols, D.S., Nichols, P.D. and McMeekin, T.A., 1993. Polyunsaturated fatty acids from Antarctic bacteria. Ant. Sci., 5: 149–160.

Nichols, D.S., Nichols, P.D. and McMeekin, T.A., 1995. A new n-$C_{31:9}$ polyene hydrocarbon from Antarctic bacteria. FEMS Microbiol. Lett., 125: 281–286.

Nichols, P.D., Holdsworth, D.G., Volkman, J.K., Daintith, M. and Allason, S., 1989. High incorporation of essential fatty acids by the rotifer *Brachionus plicatilis* fed on the Prymnesiophyte alga *Pavlova lutheri*. Aust. J. Mar. Freshw. Res., 40: 645–655.

Phillips, N.W., 1984. Role of different microbes and substrates as potential suppliers of specific essential nutrients to marine detrivores. Bull. Mar. Sci., 35: 283–298.

Rieper, M., 1978. Bacteria as food for marine harpaticoid copepods. Mar. Biol., 45: 337–345.

Rimmer, M.A., Reed, A.W., Levitt, M.S. and Lisle, A.T., 1994. Effects of nutritional enhancement of live food organisms on growth and survival of barramundi/sea bass *Lates calcarifer* (Bloch) larvae. Aquacult. Fish. Manage., 25: 143–156.

Southgate, P.C. and Lou, D.C., 1995. Improving the n−3 HUFA composition of *Artemia* using microcapsules containing marine oils. Aquaculture, 134: 91–99.

Volkman, J.K., Jeffery, S.W., Nichols, P.D., Rogers, G.I. and Garland, C.D., 1989. Fatty acid and lipid composition of 10 species of microalgae used in mariculture. J. Exp. Mar. Biol. Ecol., 128: 219–240.

Watanabe, K., Sezaki, K., Yazawa, K. and Hino, A., 1992. Nutritive fortification of the rotifer *Brachionus plicatilis* with eicosapentaenoic acid-producing bacteria. Nippon Suisan Gakkaishi, 58: 271–276.

White, D.C., Davis, W.M., Nickels, J.S., King, J.D. and Bobbie, R.J., 1979. Determination of the sedimentary microbial biomass by extractible lipid phosphate. Oecologia, 40: 51–62.

Whyte, J.N.C. and Nagata, W.D., 1990. Carbohydrate and fatty acid composition of the rotifer *Brachionus plicatilis* fed monospecific diets of yeast or phytoplankton. Aquaculture, 89: 263–272.

Yazawa, K., Araki, K., Okazaki, N., Watanabe, K., Ishikawa, C., Inoue, A., Numao, N. and Kondo, K., 1988. Production of eicosapentaenoic acid by marine bacteria. J. Biochem., 103: 5–7.

Yazawa, K., Watanabe, K., Ishikawa, C., Kondo, K. and Kimura, S., 1992. Production of eicosapentaenoic acid from marine bacteria. In: D.J. Kyle and C. Ratledge (Editors), Industrial Application of Single Cell Oils. American Oil Chemists Society, Champaign, IL, pp. 29–52.

Zobell, C.E., 1946. Marine Microbiology. Chronica Botanica, Waltham, 240 pp.

Novel Bacteria as Alternative Sources of Polyunsaturated Fatty Acids for Use in Aquaculture and Other Industries

Peter D. Nichols[1,2], David S. Nichols[2], Tom Lewis[2], John P. Bowman[2], Janelle Brown[2], Jennifer Skerratt[2] and Tom A. McMeekin[2]

[1]CSIRO Division of Marine Research, GPO Box 1538, Hobart, Tasmania 7001, Australia
[2]Antarctic CRC & Department of Agricultural Science, Univ. of Tasmania, GPO Box 252-54, Tasmania 7000, Australia
Email: Peter.Nichols@marine.csiro.au

Abstract

Fish oils or the culture of microalgal species have traditionally provided industrial sources of omega-3 (ω3) polyunsaturated fatty acids (PUFA). However, fish oil sources may be unreliable with the failure or variability of various fisheries. In the future there is concern that insufficient fish oil will be available to meet the expected growing demand for ω3 oils. The culture of algae for ω3 PUFA is comparatively expensive and complicated. In comparison, the potential production of ω3 PUFA from bacteria may be cheaper and easier to produce resource. Several species of bacteria have been isolated from the Australian Antarctic Territory that are capable of producing ω3 PUFA (e.g. eicosapentaenoic acid, EPA; 20:5ω3) at similar relative levels to those found in microalgae or fish oils. Other species have been isolated that produce docosahexaenoic acid (DHA, 22:6ω3) and a mixture of EPA and arachidonic acid (AA, 20:4ω6). These bacteria may represent a potential new, cheaper and easier to produce source of ω3 and ω6 PUFA for the food, aquaculture and related industries.

Introduction

Every year, vast areas of sea-ice forms at the ocean surface surrounding Antarctica. Ice coverage may extend from around 3 million km^2 in February, to a peak of 20 million km^2 in September/October (Vincent 1988). The sea-ice supports the growth of a variety of microalgae (mostly pennate diatoms) together with an active bacterial community. This sea-ice microbial community (SIMCO) is a major component of the carbon and energy flux in the polar ocean, and is the foundation of the Antarctic food chain.

An important role of the SIMCO, with respect to higher trophic levels, is the biosynthesis of PUFA such as EPA and DHA. Only certain bacteria, together with unicellular microalgae, possess the capability to produce PUFA *de novo* (Watanabe et al. 1983; Lubzens et al. 1985). Many other marine organisms cannot synthesize these fatty acids themselves, but rely on

dietary intake of these components or their immediate precursors (Lubzens *et al.* 1985). The sea-ice microbiota is therefore an important source of these essential dietary components for many marine organisms.

This study investigates the fatty acid composition of psychrophilic sea-ice bacteria isolated from Prydz Bay. PUFA production has only been observed for a limited number of bacteria (Delong and Yayanos 1986; Yazawa et al. 1988a&b), including recently in Antarctic bacteria (Nichols et al. 1993b; Nichols & Russell 1996; Bowman et al. 1997). Results are also reported for other novel hydrocarbons present in the sea-ice bacteria, and for preliminary trials examining the use of the PUFA-containing bacteria for enriching rotifers used as live-feed for early larval stages in the aquaculture industry.

Materials and Methods

Bacterial strains used for fatty acid analysis and feeding trials were obtained from the Australian Collection of Antarctic Microorganisms (ACAM), University of Tasmania. Strains were also isolated from sea-ice cores collected in Prydz Bay and near-by lakes during the 1990/91 and 1993/94 summer seasons, as described in Nichols et al. (1993b) and unpublished data. Rotifer feeding trials using PUFA-containing bacteria were conducted using similar protocols to those outlined in Nichols et al. (1996).

Lipids from bacterial samples were extracted using the modified one-phase chloroform-methanol Bligh and Dyer extraction as described in Nichols et al. (1993a&b). For bacterial samples, a portion of the total-lipid extract was transmethylated by reaction for 1 h at 80°C with methanol-chloroform-hydrochloric acid (10:1:1, v/v/v, 3 ml). The resultant fatty acid methyl esters (FAME) were extracted with hexane-chloroform (4:1, v/v; 3 x 2 ml) (Nichols et al. 1993b). For microalgal samples, a portion of the total lipid extract was first saponified by reaction at 60°C for 3 h using 3 ml of a 5% KOH in 80% methanol (w/v) solution. Subsequent fatty acid methylation was achieved by acidification of the aqueous layer after initial extraction of the non-saponifiable neutral lipids, and extraction with hexane/chloroform (4:1 v/v, 3 x 1.5 ml). Gas chromatographic (GC) and GC-mass spectrometric analyses of FAME samples were conducted using a Hewlett Packard 5890 GC and 5970 mass selective detector (MSD) as described in Nichols et al. (1993a&b) or FISONS MD 800 GC-MS. Analysis of the polyene hydrocarbon and hydrogenation product was conducted using GC and Kratos Concept ISQ mass spectrometer as detailed in Nichols et al. (1995b).

Results and Discussion

Bacterial PUFA

Of over 110 psychrophilic bacterial strains analysed, 17 were found to produce at least trace levels of EPA. Several strains contained high levels of EPA, with one strain (ACAM 456) containing up to 17% EPA of the total fatty acids (Figure 1; see also Nichols & Russell 1996). ACAM 456 is now described as *Shewanella gelidimarina* (Bowman et al. 1997b). In comparison, sea-ice diatom communities examined contained high levels of PUFA, in the range

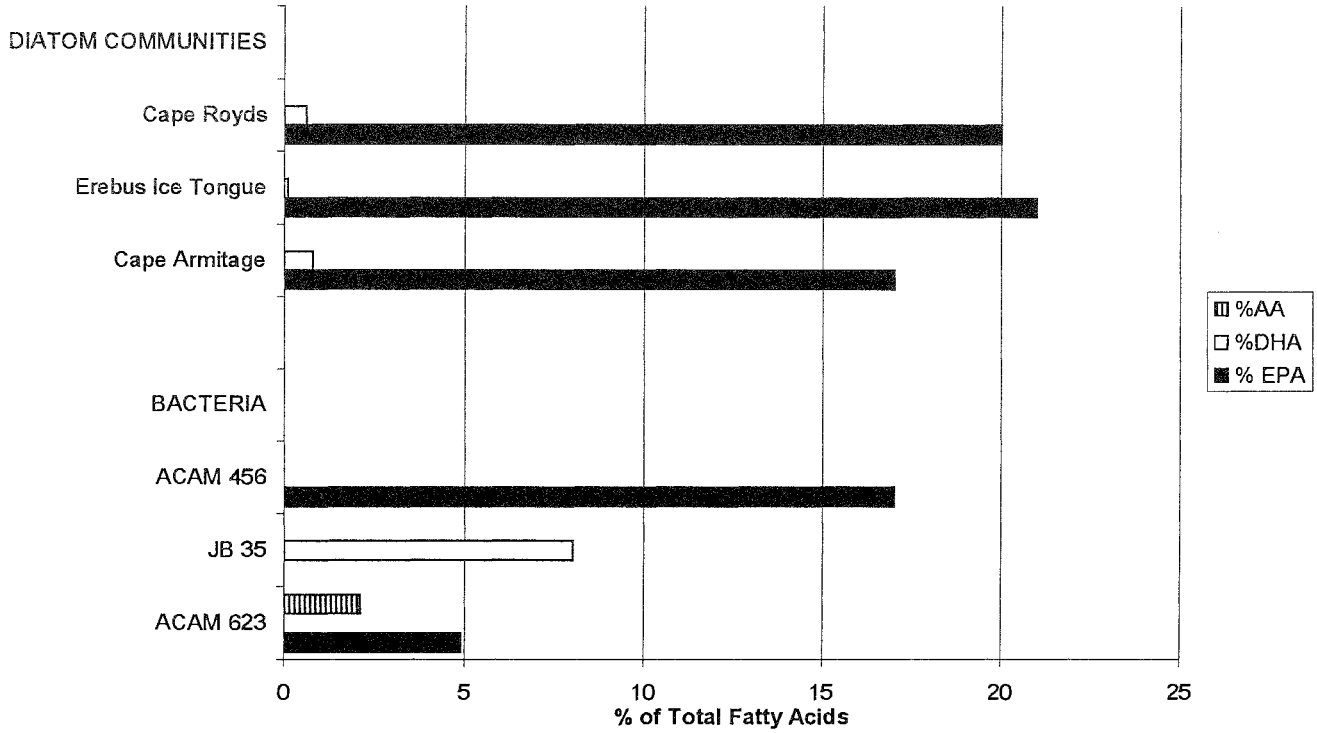


Figure 1. Eicosapentaenoic acid, docosahexaenoic acid and arachidonic acid levels (of total fatty acids) in selected Antarctic bacteria and sea-ice diatom communities. Diatom data from Nichols et al. (1993a).

of 40-50% of total fatty acids (TFA), with an average level of EPA at 19% of TFA (Figure 1). These levels are similar to or higher than those found in pure cultures of Antarctic and temperate diatom species (Whitaker & Richardson 1980; Gillan et al. 1981).

Another 16 bacterial strains produced other PUFA such as 18:2ω6, 18:3ω3 and 20:4ω3. Five strains (e.g. JB 35) produced 22:6ω3 (DHA), the first strains to do so not isolated from the deep-sea (Figure 1). Some of these strains have been recently described as *Colwellia psychroerythrus* (Bowman et al. 1997a). One other strain (ACAM 623) has been isolated that produces both EPA and AA (Figure 1) (Nichols et al. 1997). This strain produced between 4 to 15% of these two PUFA (3 to 12% EPA, 1 to 3% AA) depending on growth temperature. The proportion of EPA decreased in a linear manner with increasing growth temperature over the temperature range 2 to 15°C. In contrast the proportion of AA remained unchanged, suggesting that AA is not involved in the modulation of both lipid phase and fluidity in response to changes in temperature.

The proportion of bacterial EPA-producers found from the Antarctic sea-ice is also an order of magnitude higher than for temperate marine environments (Yazawa et al. 1998a&b) (Figure 2). This finding suggests that the physico-chemical parameters of the Antarctic sea-ice environment have selected for bacterial strains capable of maintaining a functional lipid membrane by the production of PUFA (Nichols et al. 1995a).

International interest exists in the transfer of PUFA genes to higher oil yielding microorganisms and ultimately oil seed plants. Bacterial DNA is simpler than eukaryote-derived DNA. It is likely, therefore, that bacterial DNA (e.g. from the EPA and DHA producing organisms such as *Shewanella* and *Colwellia* spp.) may be used as a source of genetic material in the future for production of EPA and DHA by transgenic microorganisms or higher plants.

Other novel lipids

A novel n-$C_{31:9}$ polyene was found in several PUFA-producing strains. To our knowledge, this is the first isolation of a hydrocarbon of this type (Nichols et al. 1995b). Although full chemical characterisation has not been completed, we suspect it is biosynthetically derived from EPA, by the head-to-head condensation of two EPA molecules, and represents a further membrane adaptation for stabilisation at low temperature.

Bacterial PUFA - Aquaculture and other applications

The possibility of using bacteria as aquaculture feeds has been previously considered, but their perceived lack of PUFA was thought to be a major drawback (Phillips 1984). It is now evident that certain strains of bacteria do produce high levels of PUFA. The ability to produce PUFA allows these bacteria to be considered as a valuable addition or alternative to current aquaculture feeds. Bacterial production of PUFA represents a renewable resource, in comparison to the variable nature of fish catches that are currently the most common source of ω-3 PUFA. The culture of bacteria is also considered far cheaper and easier than that of algae as a PUFA source. The industrial usage of purified PUFA (such as EPA or DHA) as health products, food additives or pharmaceuticals is also increasing. Bacteria possess the advantage, relative to fish oil and most algal oils, that the majority of PUFA-producing strains accumulate only one type of PUFA in particular, usually EPA or DHA. This potentially could remove the

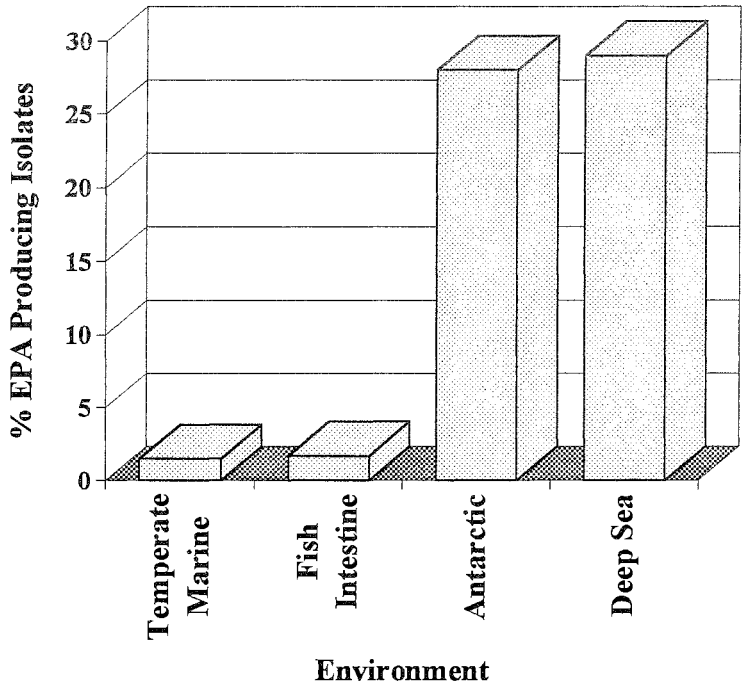


Figure 2. Proportion of EPA-producing bacteria from various environments. Data from Yazawa et al. (1988a&b).

costly exercise of purifying these individual PUFA from the more complex mixtures usually present in fish oils or algae.

Results for recent preliminary experiments examining enrichment of rotifers with both EPA and DHA are presented in Table 1, together with data for rotifers fed the green microalga *Tetraselmis suecica* or yeast. Rotifer EPA levels increased from 1 mg/g (dry weight, dw; yeast diet) to 12 mg/g dw when fed the EPA-containing bacterium (Table 1). When fed a mixture of the two bacterial strains containing EPA and DHA, final EPA and DHA levels were 5 mg/g and 3 mg/g dw respectively. Optimisation of feeding strategies is still required. However, these preliminary results demonstrate that rotifers can be enriched with both EPA and DHA from bacteria.

Microbial oils - some issues

Key issues for production of single cell oils (SCOs) from microorganisms include the current high production costs relative to fish oils and in some cases perceived toxicity problems with SCOs. Noteworthy in the latter area is the recent reporting of the arresting of embryonic development in copepods by inhibitory compounds in diatom cells, with the possibility existing that algal oil constituents may be the toxic components (Miralto et al. 1997). Similarly, microalgal-derived DHA-rich oils may contain unidentified sterol and other components. The safety of these and other unusual lipids derived from microalgal oils used for infant formulae production needs to be well established as noted at the International Meeting on Infant Nutrition held in Barcelona during late 1996 (INFORM, February 1997).

Fish oil reserves are currently considered adequate for aquaculture and other uses. There is, however, scope for better utilisation of existing fisheries, in particular through exploitation of waste or bycatch. A new initiative by Nu-Mega Lipids will soon see manufacture of the first value-added ω-3 PUFA oil products in Australia derived from fish waste. Increased demand is predicted for ω-3 PUFA containing oils rich in EPA and DHA due to the many benefits now known for such oils. Ignoring possible toxicity and related issues, the economics of obtaining SCOs presently prevents their production being economically competitive relative to fish oils. However, the knowledge that global fisheries have reached or are declining from maximum sustainable levels foreshadows increased future opportunity for the economic competitiveness of SCOs in a 5 to 10 year time frame, or perhaps for ω-3 oils derived from transgenic microorganisms or oil seed plants within 10 to 20 years.

Acknowledgments

This work was supported in part by the Fisheries Research and Development Corporation, the Antarctic Science Advisory Committee, the Australian Research Council, the British Council and Clover Corporation. We thank our many ACAM colleagues for assistance during the project. Travel support from IFREMER and The Ian Potter Foundation enabled PDN to attend and present the paper in September 1997 at the Marine Microorganisms for Industry meeting held in Brest. Dr. Jean Guezennec, IFREMER, is also thanked for organisational and other travel assistance.

Table 1. Mean eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) levels in feed bacterial cultures and rotifers fed bacteria, bacteria or yeast for 24 hours.

Sample	EPA	DHA
Bacterial strain	Percentage composition[a]	
ACAM 456	15	0
ACAM 605	1	5
Rotifer diet	mg/g (dry weight)[b]	
Starved	1	0
Tetraselmis suecica	4	0
Yeast	1	0
ACAM 456	12	0
ACAM 456+605	5	3

[a] Percent of total fatty acids

[b] Expressed as mg/g EPA or DHA of rotifer dry weight.

References

Bowman, J.P., Gosink, J.J., McCammon, S.A., Lewis, T.E., Nichols, D.S., Nichols, P.D., Skerratt, J.H., Staley, J.T. and McMeekin, T.A. (1997a) Novel *Colwellia* species isolated from Antarctic sea-ice: psychrophilic, marine bacteria with the ability to synthesise docosahexaenoic acid (22:6ω3). International Journal of Systematic Bacteriology, (submitted for publication).

Bowman, J.P., McCammon, S.A., Nichols, D.S., Skerratt, J.S., Rea, S.M., Nichols, P.D. and McMeekin, T.A. (1997b) *Shewanella gelidimarina* sp. nov. and *Shewanella frigidimarina* sp. nov. novel Antarctic species with the ability to produce eicosapentaenoic acid (20:5ω3) and grow anaerobically by dissimilatory Fe(III) reduction. International Journal of Systematic Bacteriology, 47, 1040-1047.

DeLong, E.F. and Yayanos, A.A. (1986) Biochemical function and ecological significance of novel bacterial lipids in deep-sea prokaryotes, Applied and Environmental Microbiology, 51, 730-737.

Gillan, F.T., McFadden, G.I., Wetherbee, R. and Johns, R.B. (1981) Sterols and fatty acids of an Antarctic sea-ice diatom *Stauroneis amphioxys*. Phytochemistry, 20, 1935-1937.

Lubzens, E., Marks, A. & Tietz, A. (1985) De novo synthesis of fatty acids in the rotifer *Brachionus plicatilis*. Aquaculture, 47, 27-37.

Miralto, A., Ianora, A., Poulet, S. A., Romano, G., Buttino, I. and Scala, S. (1997) Embryonic development in copepods is arrested by inhibitory compunds in diatom cells. 4th International Marine Biotechnology Conference, Italy, September 1997, abstracts, p79.

Nichols, D.S. and Russell, N.J. (1996) Fatty acid adaptation in an Antarctic bacterium – changes in primer utilisation. Microbiology, 142, 747-754.

Nichols, D.S., Nichols, P.D. and Sullivan, C.W. (1993a) Fatty acid, sterol and hydrocarbon composition of Antarctic sea-ice diatom communities during the spring bloom in McMurdo Sound. Antarctic Science, 5, 271-278.

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

Nichols, D.S., Nichols, P.D. and McMeekin, T.A. (1995a) Ecology and physiology of psychrophilic bacteria from Antarctic saline lakes and sea-ice. Science Progress, 78, 311-346.

Nichols, D.S., Nichols, P.D. and McMeekin, T.A. (1995b) A new $C_{31:9}$ polyene hydrocarbon from Antarctic bacteria. FEMS Microbiology Letters, 125, 281-286.

Nichols, D. S., Hart, P., Nichols, P. D. and McMeekin, T. A. (1996) Enrichment of the rotifer *Brachionus plicatilus* fed an Antarctic bacterium containing polyunsaturated fatty acids. Aquaculture, 147, 115-125.

Nichols, D. S., Brown, J., Nichols, P. and McMeekin, T. A. (1997) Production of eicosapentaenoic and arachidonic acids by an Antarctic bacterium: Response to growth temperature. FEMS Letters, 152, 349-354.

Phillips, N.W. (1984) Role of different microbes and substrates as potential suppliers of specific essential nutrients to marine detrivores. Bulletin of Marine Science, 35, 283-298.

Vincent, W.F. (1988). Microbial ecosystems of Antarctica Cambridge: Cambridge University Press, 304 pp.

Watanabe, T., Kitajima, C. and Fujita, S. (1983) Nutritional values of live organisms used in Japan for mass propagation of fish: A Review. Aquaculture 34, 115-143.

Whitaker, T.M. and Richardson, M.G. (1980) Morphology and chemical composition of a natural population of an ice-associated Antarctic diatom *Navicula glaciei*. Journal of Phycology, 16, 250-257.

Yazawa, K., Araki, Okazaki, N., Watanabe, K., Ishikawa, C., Inoue, A., Numao, N. and Kondo, K. (1988a) Production of eicosapentaenoic acid by marine bacteria, Journal of Biochemistry, 103, 5-7.

Yazawa, K., Araki, K., Okazaki, N., Watanabe, K., Ishikawa, C., Inoue, A., Numao, N., Kondo, K., Watabe, S. and Hashimoto, K. (1988b) Eicosapentaenoic acid productivity of the bacteria isolated from fish intestines, Nippon Suisan Gakkaishi, 54, 1835-1838.

MARINE BIOLOGICS
Incorporated

$72
P. NICHOLS
M. BAKES

July 19, 1996

Dear Dr. Fandry,

The highlight of our recent trip to the South Pacific was our visit to the CSIRO Division of Oceanography!

You and your associates are really to be commended for the marine science that emanates from those beautiful laboratories.

Aside from the pure science activities I was really impressed with the high morale of your staff and the unanimous respect they each had for you as an individual and the leadership qualities you display in the coordination of your large facility.

My only regret is that I could not have remained a few days longer as I was receiving a meaningful education in our field of deep water shark liver oil. We were particularly grateful for the considerate treatment and meaningful education we received from Dr. Peter Nichols....he is a real credit to your organization as is Dr. Michael Bakes.

Again, Dr. Fandry, we thank you for your hospitality and will look forward to an opportunity to visit you again.

Best regards,

Floyd E. Weston, Ph.D.
President

Dr. Chris Fandry
CSIRO Division of Oceanography
GPO Box 1538
Hobart Tasmania
7001 Australia

FEW/cu

1955 South Ridgewood Way • Bountiful, Utah 84010 • (801) 294-8610 • Fax (801) 294-6906

SQUALUS

November 18, 1993

Dr Peter Nicols
Division of Oceanography
CSIRO
Castray Esplanade
HOBART Tas 7001

Dear Dr Nicols,

re: FRDC funding application 93/94, Marine Oil R&D

Squalus has no hesitation in supporting your request for further FRDC funding. Both Squalus and Australia already stand to benefit from you earlier work into the fractionation of shark liver oils. The research proposed in this application should further enhance the value of Australia's marine resources.

A thorough knowledge of the composition of marine oils is an important pre-requisite to the development of commercial products from the oils. Our current level of knowledge is inadequate and your proposed work should help redress this problem.

Given Australia's relatively high cost of labour it is important that we maximise labour productivity by having the best available technology. Your proposed work on supercritical fluid extraction and fractionation may significantly improve Australia's international competitive edge in the processing of marine oils.

Increasing the value-added to Australia's marine resources is important to both the industry and the nation. Your earlier work has already contributed to this goal and, we believe, that the research proposed in the application has a strong chance of further enhancing the value of our marine resources

Yours faithfully

Chris Lightfoot
Managing Director

SQUALUS PTY LTD ACN 005 762 612
6 EARLSFIELD ROAD, HAMPTON, VIC. 3188 AUSTRALIA
INTERNATIONAL TEL 613 521 0016 FAX 613 521 0075
DOMESTIC TEL (03) 521 0016 FAX (03) 521 0075

CLOVER CORPORATION PTY LTD
ACN 001 512 544
8 RODBOROUGH ROAD
FRENCHS FOREST NSW 2086
AUSTRALIA
TELEPHONE (02) 975 5733
INTERNATIONAL 61 2 975 5733
FACSIMILE (02) 975 5984
INTERNATIONAL 61 2 975 5984

22nd November, 1993.

TO WHOM IT MAY CONCERN

Re: 1993/1994 F.R.D.C. APPLICATION

We have had discussions with the C.S.I.R.O. Division of Oceanography and the work which they are doing particularly in reference to the Australian species of Fish is of extreme interest to the Food and Pharmaceutical Industry in Australia.

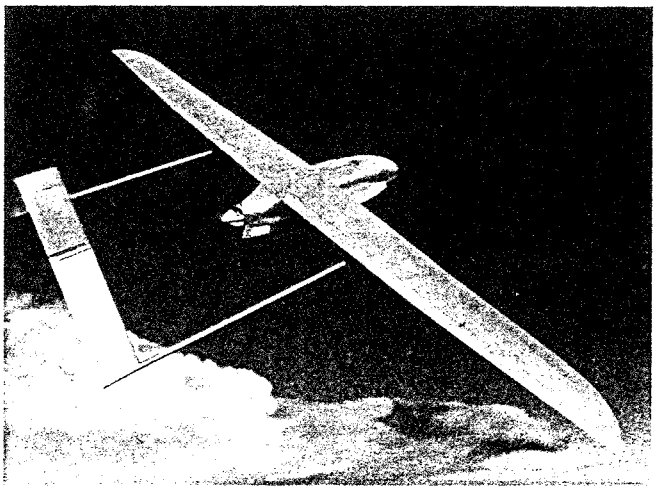
There is an increase in the knowledge and specific needs of Omega 3 Polyunsaturated Fatty Acids both in the prevention of disease and in the treatment of existing disease.

The essential part of any project will be to use functional techniques to increase the Omega 3 Fatty Acids which are present in low concentration for higher therapeutic levels and to make these fractions applicable to both the Food and Pharmaceutical areas.

For example: Baby Food Industry, Treatment of inflammation, in the Pharmaceutical Industry and for general maintenance of Omega 3 levels which are essential in everyday life.

We thoroughly support any project which will allow Australia to make use of its resources and adding value to an existing industry particularly as most world sources are contaminated by pesticide problems.

HAMISH DRUMMOND
Managing Director



Model plane to search for minerals

MODEL aircraft with wingspans of only three metres are being evaluated by the mining industry as a low-cost method of mapping mineral deposits over large areas.

The Australian Mineral Industry Research Association (AMIRA) believes the project could cut the cost of geophysical surveys from $9-15 per km to $1 per km.

Fitted with a miniature magnetometer, the craft would fly as low as 30 metres, producing maps with "exquisite detail".

The CRC for Australian Mineral Exploration is assessing the feasibility of making extensive modifications to the Aerosonde, an Australian unmanned aircraft used in full-scale flights last year by the Australian Bureau of Meteorology to study thunderstorms near Darwin.

The 14-kilogram Aerosonde has been developed collaboratively since 1992 by the Bureau of Meteorology, Perkins Engineering (owned by Australian touring car champion Larry Perkins) and the Insitu Group (a small Californian aeronautical company).

Modified for mineral exploration, it would be able to fly and land by itself, and would have a range of 3000 km. This would enable it to operate continuously for two to three days, flying in lines as close as 10m apart.

Further information:
James Macnae
02 9850 9291

ANZAAS Congress: Defending our Planet

THE 65TH Congress of the Australian and New Zealand Association for the Advancement of Science (ANZAAS) will be held in Canberra from 30 September to 3 October, with the theme "Defending our Planet".

The Congress will explore the role of science in identifying and understanding threats to sustaining human existence on Earth, and in developing and implementing defences against them.

The major sponsor is EnergyAustralia, and the Congress venue is Rydges Lakeside, Canberra.

Further information
1800 063 046

Ocean going solar boat for research

A LONG-RANGE solar-powered research vessel which would have the capacity to keep stationary in the open ocean for up to three months is being developed by engineering staff at James Cook University in Townsville.

The unmanned vessel is seen as a low-cost alternative to manned research vessels, which cost thousands of dollars a day to operate.

The proposed vessel would cost only around $10,000, but could be fitted with a range of instruments to measure ocean characteristics including currents, pollution, temperatures, wind speeds and salinity.

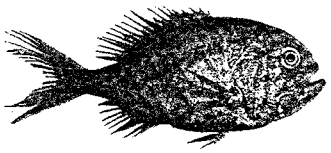
A fibreglass-hulled prototype, about 4m long, should be operational before the end of the year.

It will have Global Positioning System satellite navigation, and satellite communication.

The idea for the vessel came from a proposal to hold a solar powered boat race from New Zealand to Townsville.

Further information
Peter Grabau
077 81 5092

Orange roughy oil good for cutting tools



A FISH oil that prolongs the lifetime of high-speed metal cutting tools by between 25-300 per cent is soon to be launched by a Tasmanian company following four years of R&D.

The oil, previously a waste product from catches of orange roughy fish, is a more effective cutting fluid than conventional petrochemical-based fluids.

It is odourless, competitive in price, and can be used on all kinds of metal cutting tools including lathes, drills, saws and tappers.

Hobart-based fish oil refining company, Beku Environmental Products, has been granted international patents recognising the oil as a degreaser and cutting fluid.

Company spokesman Dan Cudmore said orange roughy-based cutting fluid would be marketed at around $6 per litre.

He said Beku had also succeeded in refining the oil even more highly into a valuable cosmetic product for which there is worldwide demand.

The unusual properties of the oil were noticed in 1989, when fishermen slipping around in fish oil all day found that it was reacting with the plastics in their boots.

Beku Environmental Products was established as a result of a 1990 collaborative research project on orange roughy oil with CSIRO.

The company also processes liver oil from deep-sea sharks, another fishing by-product that was previously discarded.

One fraction of deep sea shark liver oil, known as squalene, is sold as a health tonic in the world's human health markets. A second fraction, known as alkoxy glycerol, is reputed to boost the human immune system and has relevance in cancer treatment.

Further information:
Don Cudmore
002 73 9377

SEAFOOD & HEALTH

Heinz is chasing $400M market for "brainfood"

THE Heinz company is chasing a share of what is expected to soon be a $400-million-a-year world market for Omega 3 oil from fish.

H.J. Heinz Company Australia announced recently that it has formed a $5 million joint venture operation with research and development concern Clover Corporation.

The joint venture organisation, Nu-Mega Lipids, will use a by-product of the tuna canning process to produce high quality refined tuna oil which contains the essential Omega 3 oil, or fatty acid, DHA.

A spokesman said that this had a range of applications, including being used in the treatment of heart and other diseases and to improve a nutrition and "brain-power".

"DHA has already been found to have a positive benefit in infant brain and retina development, and in cardiovascular disease, diabetes, hypertension and thrombosis."

Joint venture General Manager Guy Drummond said Clover Corporation and the CSIRO's Divisions of Oceanography and of Human Nutrition had been exploring the health benefits of Omega 3 rich tuna oil in the battle against heart disease and in human nutrition generally.

The CSIRO has also been involved in development of the oil refining process.

"There is already widespread use of this refined tuna oil in Europe and throughout Asia, where the product is used to boost nutrition in many foods and to treat medical conditions," Mr Drummond said.

Under the joint venture, tuna oil from Heinz' canneries at Eden on the New South Wales' coast and in American Samoa will be transported to the Melbourne plant for refining and distribution to food and pharmaceutical companies.

Pharmaceutical industry experts estimate the global market for the refined tuna oil at around $A400 million within five years.

Heinz Managing Director Tony Dyson said this is a whole new business for Heinz.

"The positive health benefits of the product are in line with our aim to be Australia's Premier Family Food Company," he said.

"Environmentally, it utilises a product that otherwise would be disposed of as waste. In the long term it will create jobs and, given the quality of the tuna caught in the Pacific and Southern Oceans, we expect the

Heinz will be extracting Omega 3 oil from tuna but another prime source is mullet and two or three meals a week can help protect against a number of potential health problems.

Gold Coast meeting

This was the scene at a Gold Coast meeting called by the local Sunfish branch in early March to discuss research in the Southport Broadwater. (See the report in last month's "Queensland Fisherman".) This meeting followed calls from some members of Sunfish to ban commercial fishing in the Broadwater. The meeting attracted about 70 people (and that number included several commercial fishermen), indicating a disturbingly low level of concern about the Broadwater's environment but also little feeling against commercial fishing amongst the Gold Coast's population of some 350,000 people. It is understood that little progress has been made on the research proposal since the meeting and that the then Sunfish branch secretary, Max Moore, has since resigned from the position but will still be pursuing a research project on the Broadwater environment privately. QCFO Environment Officer Stephen Tapsall, who attended the Gold Coast meeting, expects to be liaising with him on environmental issues of common concern.

CSIRO analysing oil content of Australian fish

RESULTS are beginning to emerge from a comprehensive research project analysing the oil content of Australian fish.

CSIRO researchers are half way through the $400,000, two-year project and recently released some preliminary results.

Those involved in the project seem enthusiastic about the results and about the health benefits of Australian fish.

"When we talk about riches of the sea, this study is generating a new dimension for Australian wild fisheries and aquaculture," CSIRO researcher Patti Virtue said recently.

"There are definite trends that indicate how fish oils vary in medicinal value from season to season and between tropical and temperate regions," Dr Virtue said.

"We have also established that trash fish caught as a bycatch to other species, such as prawns, are high in valuable marine oils and have potential for that benefit alone."

The research is being jointly funded by the Fisheries Research & Development Corporation (FRDC) and the CSIRO.

The research project is being conducted by scientists from the CSIRO Marine Research laboratories in Hobart and Melbourne, together with the Division of Human Nutrition in Adelaide.

It will provide a guide for analysing and comparing polyunsaturated fatty acids (oils) in Australian fish, such as Omega 3 oil.

The National Health & Medical Research Council has recommended these polyunsaturated fatty acids should be increased in the diet of Australians.

Dr Virtue said that Australia is a leading international researcher in the marine oil field, with considerable US and European interest in the fish species database being developed as part of the study.

Provisional results she presented to the American Zoological Society recently had confirmed the level of interest in the Australian research, she said.

The CSIRO study helped pave the way for the multi-million-dollar joint venture between H.J. Heinz and the Clover Corporation to begin refining tuna oil rich in Omega 3 oil for what is expected to be a $400-million-a-year market within five years. (See adjacent story.)

Dr Virtue said the fish being examined had been supplied by State fisheries authorities from commercial catches, covering most popular fish species found in Australian fish markets and fish shops, plus other, low-value or non-commercial species, either caught in local waters or imported from overseas.

At the same time, CSIRO has started collation of a definitive guide, *Handbook of Australian Seafood: A Guide to Whole Fish & Fillets*, which she said would allow commercial fishermen, retailers and consumers to learn more about the fish they are catching or buying.

joint venture to be the leading global producer of refined tuna oil."

The project also has the backing of the Victorian Government.

"Nu-Mega Lipids is the type of development we would like to see more of in Victoria," Industry, Science & Technology Minister Mark Birrell said.

"It has harnessed local research and technical know-how, is value-adding to a natural resource, has the potential to boost human nutrition and the treatment of disease, and is opening the door to export markets."

Production of the refined tuna oil was expected to begin shortly.

Facts about fish oil

Nu-Mega Lipids General Manager Guy Drummond explained that the fish oil had a number of applications in health.

He said that fat is essential in the diet in small amounts as:

1. storage of energy as body fat;
2. an essential part of the structure of the membrane of body cells;
3. a precursor of hormones which regulate the body's activity and autacoids, human-like substances which regulate body function.

Fatty acids are part of the structure of a fat and affect its function.

Fatty acids are classified as:

1. Saturated Fatty Acids (SFA)
2. Monosaturated Fatty Acids (MUFA)
3. Polyunsaturated Acids (PUFA)

Fish oils provide the human body with essential PUFA.

Omega 6 and Omega 3 fatty acids are essential PUFA, which the body can't make.

Alpha-lenolenic acid is an omega 3 fatty acid and it is converted to eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) in the body, but Omega 6 and Omega 3 fatty acids compete for the same enzymes to be metabolised, so directly supplying EPA or DHA to the body ensure that these health protecting fatty acids are available.

Nutritional Benefits

In babies, DHA is found in the grey matter in the brain and in the retina of the eye. It is essential for normal neural development. Breast milk contains DHA.

Breast milk and fish oils contain EPA and DHA as such, otherwise the body has to convert alpha-lenolenic acid and compete for its conversion with the Omega 6s.

In adults, fish oils directly provide EPA and DHA that can:

- reduce elevated triglyceride (blood fat) levels;
- reduce cholesterol levels in some cases;
- increase the ratio of HDL/LDL cholesterol;
- reduce atherosclerosis;
- reduce blood clotting;
- reduce blood pressure;
- have some anti-inflammatory effects;
- protect against bowel cancer;
- provide EPA and DHA to the aged.

"Nu-Mega Lipids can now refine fish oil to be used in a variety of foods such as margarines, salad dressings, baby foods, milk drinks, etcetera so that the benefits of EPA and DHA are available in people's food supply," he said.

Editor's note: The Omega 3 oils, or fatty acids, DHA and EPA, are available in most Australian fish in sufficient quantities to provide a health benefit from eating two or three main meals of fish per week.

Fish oil to earn millions

By ELIZABETH STACEY

TASMANIA could become a major name in the United States health-products market thanks to a lucrative new fish-oil deal.

Worth close to $2.5 million, the five-year deal between Goodwood company Beku Environmental Products Ltd and US company Oceania Products Ltd is for at least 85 tonnes of the raw fish-oil product alkoxyglycerol to be exported to Canada, the US and Mexico.

Dan Cudmore: new deal opens other doors.

Found in the livers of deep-sea sharks — called squalidae — alkoxyglycerols are reputed to enhance the body's immune system and to diminish the potential damage caused by radiotherapy treatment for cancer.

The sharks are caught as a bi-product of fishing for orange roughy.

Once the product is turned into capsules in the US, it will be marketed to 600 medical clinics and will bring in about A$85 million in retail sales.

Beku marketing manager Dan Cudmore said the deal, which was announced yesterday at Tasmania Development Resources Hobart office, was a major coup for the five-year old company, which employs seven people.

"This is a very important time for Beku," he said. "We have always identified this product as having potential."

Mr Cudmore said the deal expanded the company's market potential, which previously had been primarily Asian.

It also opened other doors for Tasmania — such as the sale of oils from salmon and for ling liver oils.

He said Tasmania was ahead of other countries in this area.

"The key elements in winning this contract were Beku's ability to purify the oil at a level higher than that being achieved elsewhere, the fact the resource is drawn from highly prized environmentally clean southern oceans, and the support and association of the CSIRO," Mr Cudmore said.

CSIRO Marine Division marine products project leader Peter Nichols said Tasmania's clean waters were essential to the success of such products.

He said investigations were also being made into utilising other products from the small sharks used to make alkoxyglycerols.

The product is expected to be ready for sale on the US market in December.

US to get our shark liver oil

Five-year contract

A Tasmanian company has won a multi-million dollar contract to supply shark liver oil to North America.

Beku Environmental Products Ltd, of Hobart, yesterday revealed it had signed the exclusive five-year contract, worth about $2.5 million, with Oceania Products Ltd, of the US.

The oil, from a small deep sea shark caught in Southern waters, will be used in health and medical products. Under the contract agreement, Beku will supply 85 tonnes of the oil, equivalent to 170 million capsules and worth $85 million.

Beku marketing director Dan Cudmore yesterday said that the company, which now employs seven people at its Goodwood plant, expected the market to expand dramatically because of the high quality of the oil.

"Our catch comes from the Great Australian Bight, the Tasman Sea and the Southern Ocean and is regarded as the purest in the world," Mr Cudmore said.

He said that oil from the other major source, the North Atlantic and Scandinavian waters, was showing increasing levels of contamination from pollution.

The oil is synthensised to produce high levels of alkoxylglycerol, which enhances the human immune defence system and is believed to reduce potential damage from radiotherapy for cancer sufferers.

Six species of deep water shark can be used to produce the oil. The sharks average less than 1.5 metres in length but their livers make up 20 per cent of total body weight and can weigh several kilograms.

The sharks share the same water depths as the orange roughy. In the past, thousands of sharks killed by the pressure changes in being brought to the surface in the orange roughy nets have been regarded as waste and thrown overboard.

Mr Cudmore said that their value now was recognised and some fishing boats were now seeking them exclusively. But he said the numbers needed to meet Beku's requirements made up only a tiny fraction of the resource, which was being monitored by the CSIRO.

"All the studies so far have shown the resource to be totally sustainable," he said.

The Advocate, Thursday, November 7, 1996

$85m. purified shark oil contract

By Michelle Grima

A TASMANIAN marine oil processor has secured an $85m. contract coup after signing an exclusive supply agreement with an American company yesterday.

The five-year contract will mean Beku Environmental Products Ltd, of Goodwood, will supply specialised human health product derived from fish oil to US company Oceana Products.

The first 1.3 tonnes of the product has already been airfreighted to the US and bulk shipments will continue to leave from Hobart.

It will then be encapsulated and marketed to more than 600 medical clinics throughout North America.

The product — a deep sea shark liver oil fraction — enhances the human immune defence system by increasing white blood cells and is said to diminish potential radiotherapy damage in cancer therapies.

Beku marketing director Dan Cudmore said the key elements in winning the contract were the company's ability to purify the oil to a level higher than anywhere else in the world.

Beku has worked in conjunction with the CSIRO in Hobart to develop the oil, led by Dr Peter Nichols.

DAILY COMM NEWS
November 8, 1996
Page
11

Shark oil exports a boost for Tas

HOBART: Oil from deep-sea shark livers believed to enhance the human immune system is set to be an expanding export industry for Tasmania – but you can't buy it in Australia.

Beku Environmental Products Ltd has just signed a five-year contract with an American company to market $85 million worth of the oil in the United States, Canada and Mexico.

Although stopping short of claiming the oil was akin to a miracle cure, Beku marketing director Dan Cudmore said studies found the alkylglycerol it contained could boost the human immune system by increasing white blood cells and thrombocytes.

Alkylglycerol was also believed to have the potential to reduce the effects of radiation therapy in cancer patients.

Mr Cudmore said the product, which had been developed in consultation with CSIRO's Marine Division here, was not available for general sale in Australia.

Although shark liver oil had been consumed for centuries, the Therapeutic Goods Administration had not released the product for general consumption.

"You can't list that product for Australian sales and have it generally sold," Mr Cudmore said.

"You can list it for production in Australia as long as you export it.

"It's not quite a Lorenzo's Oil this product, but we would receive six to eight phone calls a week from people around Australia who hear about this product.

"They have someone or they know someone who's got cancer or who's undergoing chemotherapy and they would love to get hold of this product."

As Beku exports the oil rather than the capsules sold at a retail level, it had made no application to have the product listed other than for export.

Mr Cudmore doubted whether the American company would apply either given the size of the potential market in Australia and the fact that the product was unable to be patented because it was a natural oil.

A minimum 85 tonnes of the oil, concentrated to a level not achieved elsewhere, will be exported under the five-year deal.

The clean waters of the Southern Ocean were the key to marketing fish oil overseas.

A Greenpeace report into items produced from fish from waters in the northern hemisphere found that more than 80 per cent contained organochlorins, CSIRO marine products project leader Peter Nichols said.

"Our waters are pristine," Dr Nichols said.

"We do not have pollutant problems and that is a key marketing feature."

The species of shark used to make the oil is a small variety found only in deep waters, usually caught in orange roughy and other deep-sea fisheries.

Until the useful properties of its liver oil were discovered, the shark were discarded.

AAP

Nutritional analysis of the flesh and oil of yolla, the Tasmanian mutton bird *Puffinus tenuirostris*: a useful source of omega-3 polyunsaturated fatty acids

David R. Woodward, Malcolm D. Riley, Donald R. Buick, David S. Nichols, Peter D. Nichols, in collaboration with the Tasmanian Aboriginal Centre

(see commentary, pages 54–55)

Abstract The chemical composition of the cooked skinless flesh and the proventricular (stomach) oil of the mutton bird ('yolla') has been investigated. The flesh has high levels of vitamin A, iron, zinc, calcium, iodine and selenium. Its fat (18.7 g per 100 g flesh) has a high proportion of omega-3 polyunsaturated fatty acids (9.3% of total fatty acids). The oil, which comprises 64% waxes and 32% triglycerides, is similarly rich in omega-3 fatty acids (24.9% of total fatty acids). The amounts are such that a typical Australian's intake of omega-3 fatty acids would be at least doubled by consuming 100 g of the flesh per week, or 1 g oil per day. The available and potential supply of mutton bird flesh and oil is substantial. For those who consume it regularly, the flesh may assist in primary prevention of cardiovascular disease. Regular consumption may also assist in the management of individuals with atherogenic and thrombogenic conditions. The oil may have therapeutic usefulness for atherosclerotic and thrombotic conditions; however, the bioavailability of the wax fatty acids have not been determined. It could also have potential in the mariculture industry. (**Aust J Nutr Diet** 1995;52:87–91).

Keywords: mutton bird, yolla, *Puffinus tenuirostris*, Aboriginals, chemical analysis, omega-3 fatty acids.

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 species from northern Australia. 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 appear to contain significant amounts of the 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 South Australian (SA) branch of the Australian Government Analytical Laboratories (AGAL).

For analysis, they were thawed, browned in their own fat and braised in tap water until the flesh was tender (about two hours). 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 commenced within 24 hours 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, in the New South Wales (NSW) Regional Laboratory of AGAL.

Vitamins

Thiamin was extracted by acid and enzyme digestion, separated by high pressure liquid chromatography, and assayed fluorometrically as thiochrome after post-column oxidation by potassium ferricyanide (10). Niacin was determined colourimetrically by the König 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

University of Tasmania, Hobart
Dept of Biochemistry
D.R. Woodward, BSc, PhD, Senior Lecturer
Menzies Centre for Population Health Research
M.D. Riley, BSc(hons), DipNutrDiet, Research Dietitian
Australian Government Analytical Laboratories, SA Regional Laboratory, Seaton, South Australia
D.R. Buick, BSc, GradDipBusAdmin, AAIFST, Principal Chemist
CSIRO Division of Oceanography, Hobart, Tasmania
D.S. Nichols, BSc(hons), Experimental Officer
P.D. Nichols, BSc(hons), PhD, FRACI, Principal Research Scientist
Tasmanian Aboriginal Centre, 198 Elizabeth St, Hobart
Correspondence: D.R. Woodward, Dept of Biochemistry, University of Tasmania, GPO Box 252C, Hobart, Tas 7001

under subdued light using petroleum ether, after preliminary hydrolysis with alcoholic potassium hydroxide. The extracts were taken to near dryness with nitrogen gas, redissolved in methanol and subjected to reverse phase high pressure liquid chromaography (HPLC) (C18 Nova Pak column, Waters 490 uv/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 12 m non-polar bonded phase capillary column (SGE, 12QC2/BP1).

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 25 m polar bonded phase capillary column (SGE, 25Q2/BP225). Individual fatty acids were identified by comparing retention time data with data for a range of authentic standards. The identity of several fatty acids was independently confirmed by gas chromatography-mass spectrometry on a Hewlett Packard 5890 gas chromatograph fitted with a 5971 mass-selective detector.

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°C, two hours), were analysed by gas chromatography, with a 50 m cross-linked methyl silicone capillary column (Hewlett-Packard, HP1), flame ionisation detector and a split/splitless injector, using hydrogen as carrier gas (13). Individual fatty acids were identified by comparing retention time data with data for a range of authentic standards. Identifications were independently confirmed by gas chromatography-mass spectrometry (13).

Indices of atherogenicity and thrombogenicity

Ulbricht and Southgate (14) have proposed indices of atherogenicity and thrombogenicity for foods, based on their fatty acid composition. The index of atherogenicity is given by

$$\frac{S_{12} + 4xS_{14} + S_{16}}{M + P_{n3} + P_{n6}}$$

and the index of thrombogenicity by

$$\frac{S_{14} + S_{16} + S_{18}}{0.5 \times M + 0.5 \times P_{n6} + 3 \times P_{n3} + (P_{n3}/P_{n6})}$$

In these formulas, S_x indicates the concentration of specific saturated fatty acids: S_{12} laurate (C12:0); S_{14} myristate (C14:0); S_{16} palmitate (C16:0); S_{18} stearate (C18:0). M indicates the total concentration of mono-unsaturated fatty acids; P_{n6} of omega-6 polyunsaturates; P_{n3} of omega-3 polyunsaturates.

The application of these formulas to mutton bird oil was complicated by the fact that waxes rather than triglycerides were the major lipid species, and the oil therefore contained substantial amounts of aliphatic alcohols. It was assumed, in the absence of detailed evidence, that all fatty acids (whether in waxes or triglycerides) were equally bioavailable, but that aliphatic alcohols did not share the activity (in regard to athero- and thrombo-genesis) of their fatty acid relatives.

Results and discussion

Composition of the flesh

The mutton birds, as sold, have an average weight of about 160 g (range 130–200 g). They comprise approximately 38% inedible material, 11% visible fat, and 51% lean meat (based on dissection of four raw frozen carcases.)

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 data on the composition of mutton bird flesh presented here are the first to be published.

Compared with other Australian flesh foods (e.g. lean beef, skinless chicken, or steamed fish), mutton bird flesh is rich in vitamin A, iron, calcium and zinc (7). While

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

Energy	
kJ	1100
Macronutrients	
Protein (g)	23.9
Fat (g)	18.7
Carbohydrate (g)	0.0[a]
Vitamins	
Retinol (mg)	0.45
Beta-carotene (mg)	0.0
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

(a) Carbohydrate was determined by difference as 1.3 g/100 g. This value probably reflects accumulated errors in analysis of other components. By analogy with other flesh foods, we believe that the true value is likely to be zero, as shown in the Table.

Table 2. Fatty acid profile of skinless mutton bird flesh lipid [a,b]

Fatty acid	*Percentage of total fatty acids*
Saturated	
14:0	2.5
15:0	0.3
16:0	18.1
17:0	0.2
18:0	5.1
20:0	0.3
Total saturated	26.5
Mono-unsaturated	
14:1	0.2
16:1	8.0
18:1	36.7
20:1	8.8
22:1	6.6
24:1	0.6
Total mono-unsaturated	60.9
Polyunsaturated omega-3	
20:3	0.2
20:5	3.3
22:5	0.6
22:6	5.2
Total omega-3	9.3
Polyunsaturated omega-6	
18:2	2.3
20:2	0.2
20:4	0.8
Total omega-6	3.3
Total polyunsaturated	**12.6**

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

(b) Assuming fatty acids account for approximately 95% of total fat (a conventional value for poultry (30)), 100 g of the flesh would provide 4.7 g of saturated fatty acids. 10.8 g of mono-unsaturated, 1.7 g omega-3 and 0.6 g omega-6 polyunsaturated.

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 dietary intake (RDI) for selenium, and 180% of his iodine RDI (15). A minor disadvantage is the sodium to potassium (molar) ratio of 1.3, which is a little higher than the desirable value of less than 1.0 (16,17).

The fat content of the flesh is high (18.7%), but its PMS (polyunsaturated:monounsaturated:saturated) ratio of 0.47:2.31:1.00, is more favourable than most other flesh foods (7). Ulbricht and Southgate's indices (14), which weight fatty acids according to their cardiovascular effects (see Methods), 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 polyunsaturated fat is mainly of the C-20 and C-22 omega-3 types (normally found only to any useful extent in seafood (18)) which retard thrombogenesis and lower blood pressure (14,19,20). We estimate (Table 2) that 100 g of the flesh would provide 1.7 g omega-3 polyunsaturated fatty acids. The average Australian consumes 100 to 250 mg omega-3 fatty acids per day (20), although a recent study suggests this estimate may be somewhat high (21). Consumption of a mere 100 g per week of mutton bird flesh would provide approximately 1700 mg omega-3 fatty acids per week, equivalent to over 200 mg per day, enough to at least double current intakes.

The high content of mono-unsaturated fats, which appear to have beneficial effects on serum cholesterol (14,20,22), is also worth noting. However, consumption of 100 g mutton bird flesh (containing about 10.8 g mono-unsaturated fatty acids, Table 2) per week would have a trivial impact (about 1.5 g/d) on mono-unsaturate intakes, which are estimated as 41 g per day for men and 28 g per day for women (23).

Thus, the skinless flesh offers substantial nutritional benefits. In particular, because of its fat composition, regular consumption could 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 (20) apply equally for mutton bird.

However, our nutritional analysis and discussion are based on skinless birds, cooked without added fat, and not soaked in brine. Different preparation procedures, that involve adding saturated fats or salt, may significantly reduce nutritional desirability.

Composition of the oil

The proventricular oil comprised 32% triglycerides and 64% waxes, with small amounts of free fatty acids, polar lipid, sterols and alcohol. Table 3 lists the fatty acid and fatty alcohol profiles of the oil (the alcohols deriving from the waxes). Over 80% of the fatty acids present are either mono-unsaturates or omega-3 polyunsaturates. As fatty acids comprise in total 65.6% of the weight of the oil, 100 g of the oil would provide 6.6 g saturated fatty acids, 40.0 g mono-unsaturates, 16.1 g omega-3 and 2.1 g omega-6 polyunsaturates. The fatty alcohols are mainly saturated.

The fatty acid profile of the oil has been reported previously (24,25), but those studies used older, lower-resolution techniques. To the extent that comparisons are possible, the present and previous results are reasonably consistent.

The oil, which has a different anatomical origin to the flesh, 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 provide approximately 160 mg omega-3 and 400 mg mono-unsaturated fatty acids per day, thereby at least doubling a typical Australian's current intake of omega-3 fatty acids, though having only a trivial effect on intakes of mono-unsaturated fatty acids.

Thus, the oil may be suitable as a dietary supplement for those with atherosclerotic or thrombotic conditions,

Table 3. Fatty acid and fatty alcohol profile of mutton bird oil[a]

Aliphatic chain	*Percentage of total fatty acids* [b]	*Percentage of total fatty alcohols*
Saturated		
14:0	3.3	10.7
16:0	5.6	45.7
17:0	0.2	0.6
18:0	0.8	3.8
20:0	-	0.4
Total saturated	10.0	61.5
Mono-unsaturated		
14:1	0.2[c]	-
16:1	15.7	6.3[d]
17:1	0.7	-
18:1	36.3	10.6
20:1	3.3	9.3
22:1	3.8	10.0
24:1	0.8	2.3
Total mono-unsaturated	60.9	38.5
Polyunsaturated, omega-3		
18:3	0.7	-
18:4	2.3	-
20:4	0.7	-
20:5	12.5	-
22:5	0.5	-
22:6	7.8	-
24:1	0.8	-
Total omega-3	24.5	-
Polyunsaturated, omega-6		
18:2	2.2	-
20:2	0.2	-
20:3	0.3	-
20:4	0.5	-
Total omega-6	3.2	
Polyunsaturated, other		
16:4, *omega-1*	0.7	-
16.3, omega-4	1.0	-
Total 'other' polyunsaturates	1.7	-
Total polyunsaturated	29.2	-

(a) See note (a), Table 2.
(b) Fatty acids in total comprise 65.6 g per 100 g oil. Hence, 100 g of the oil would provide 6.6 g saturated fatty acids, 40.0 g mono-unsaturates, 16.1 g omega-3 and 2.1 g omega-6 polyunsaturates.
(c) Structural specifications of mono-unsaturated fatty acids: 14:1 comprised only omega-5 *cis*; 16:1 comprised omega-7 *cis* 15.5 and omega-5 *cis* 0.2; 17:1, omega-8 *cis*; 18:1, omega-9 *cis* 30.9, omega-7 *cis* 4.8, and omega-5 *cis* 0.6; 20:1, omega-9 *cis* 2.6, and undetermined (but not omega-7 *cis*) 0.7; 22:1, omega-11 *cis* 2.9, and omega-11 *trans* 0.9; 24:1, double bond positions not determined.
(d) Structural specifications of mono-unsaturated fatty alcohols: 16:1, double bond position undetermined; 18:1, omega-9 *cis* 5.4, omega-7 *cis* 3.8, and not determined 1.4; 20:1, omega-9 *cis* 8.6, omega-7 *cis* 0.5, and undetermined 0.2; 22:1, omega-11 *cis* 6.5, omega-9 *cis* 3.1, omega-7 *cis* 0.4; 24:1, double bond position not determined.

perhaps administered in capsule form. We estimate that 1 g of the oil would provide about 100 mg of eicosapentaenoate (EPA, C20:5, omega-3) and 60 mg docosahexaenoate (DHA, C22:6). This compares quite favourably with currently-used fish oil supplements, e.g. Bioglan Maxepa 1000 mg capsules (Rhone-Poulenc), which have 180 mg EPA and 120 mg DHA (26).

However, much of the oil comprises wax esters, which may not be hydrolysed fully in the gut of mammals such as humans (27). The bioavailability of wax fatty acids may therefore be less than 100%. Theoretically, the wax esters might produce mild steatorrhoea, with wax being excreted unchanged: however, this is unlikely to cause obvious symptoms at a 1 g dose.

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.

Adequacy of the supply

In recent years, around 200 000 to 300 000 mutton bird chicks have been harvested per annum in the Bass Strait islands (27,28). This yield would allow consumption of one bird per week (approx 100 g flesh) by several thousand people. The annual yield of around 3000 L oil (27), if used therapeutically at a dose of 1 g per day, would allow therapy for several thousand patients for a year.

However, these are minimum estimates, because it would appear feasible substantially to increase current yield, given increased demand (27,28). 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 three-fold increase in the current level of mutton bird harvesting would not threaten the long-term survival of the species (28). Increased harvesting could improve the economic outlook for the Tasmanian Aboriginal community, for whom the industry is a major employment opportunity.

Acknowledgments

The authors thank the Tasmanian Aboriginal Centre for funding the analyses. P.D.N. and D.S.N. acknowledge support from Fisheries Research and Development Corporation (FRDC). M.D.R. acknowledges a research fellowship from the Public Health Research and Development Committee (PHRDC). We thank Trevor Beard (Menzies Centre for Population Health Research), and Irynej Skira (Department of Parks Wildlife and Heritage, Hobart) for helpful advice on various points, and two anonymous Journal referees for several very constructive comments.

References

1. Brand JC, James KW, Maggiore PMA. Tables of composition of Australian Aboriginal foods. Canberra: Aboriginal Studies Press, 1993.
2. Skira I. Human exploitation of the short-tailed shearwater (*Puffinus tenuirostris*). Papers Proc Roy Soc Tas 1990;124:77–90.
3. Woodward DR, Macphail MK, King H. A history of the diet and health of Tasmanian Aborigines. In: King H, ed. Epidemiology in Tasmania. Canberra: Broiga Press, 1987: 233–58.
4. Plomley NJB. A wordlist of the Tasmanian Aboriginal languages. Launceston: the author, 1977.

5. Warham J. The incidence, functions and ecological significance of petrel stomach oils. Proc N Z Ecol Soc 1977;24:84–93.
6. Greenfield H, Southgate DAT. Food composition data—production, management and use. London: Elsevier, 1992:56–65.
7. English R, Lewis J. Nutritional values of Australian foods. Canberra: Australian Government Publishing Service, 1991.
8. Association of Official Analytical Chemists. Official Methods of Analysis, 14th edition. Washington: AOAC, 1984.
9. National Food Authority. Australian Food Standards Code. Canberra: Australian Government Publishing Service, 1992.
10. Wimalasiri P, Wills RBH. Simultaneous analysis of thiamin and riboflavin in foods by high performance liquid chromatography. J Chromatog 1985;318:412–6.
11. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959;37:911–7.
12. Volkman JK, Nichols PD. Applications of thin-layer chromatography-flame ionization detection to the analysis of lipids and pollutants in marine and environmental samples. J Planar Chromatog—Modern TLC 1991;4:19–26.
13. Nichols DS, Nichols PD, McMeekin TA. Anaerobic production of polyunsaturated fatty acids by *Shewanella putrefaciens* ACAM 342. FEMS Microbiol Lett 1992;98:117–22.
14. Ulbricht TLV, Southgate DAT. Coronary heart disease: seven dietary factors. Lancet 1991;338:985–92.
15. National Health and Medical Research Council. Recommended dietary intakes for use in Australia. Canberra: Australian Government Publishing Service, 1991.
16. Stamler J, Rose G, Stamler R, Elliott P, Dyer A, Marmot M. INTERSALT study findings: public health and medical care implications. Hypertension 1989;14:570–7.
17. Truswell AS. Potassium (K). J Food Nutr 1982;39:186–93.
18. Sinclair AJ, Dunstan GA, Naughton JM, Sanigorski AJ, O'Dea K. The lipid content and fatty acid composition of commercial marine and freshwater fish and molluscs from temperate Australian waters. Aust J Nutr Diet 1992;49:77–83.
19. Kinsella JE, Lokesh B, Stone RA. Dietary n-3 polyunsaturated fatty acids and amelioration of cardiovascular disease: possible mechanisms. Am J Clin Nutr 1990;52:1–28.
20. National Health and Medical Research Council. The role of polyunsaturated fats in the Australian diet. Canberra: Australian Government Publishing Service, 1991.
21. Sinclair AJ, O'Dea K, Johnson L. Estimation of the n-3 PUFA status in a group of urban Australians by the analysis of plasma phospholipid fatty acids. Aust J Nutr Diet 1994;51:53–6.
22. Shrapnel WS, Calvert DG, Nestel PJ, Truswell AS. Diet and coronary heart disease. Med J Aust 1992;156(Suppl):9–16.
23. English R, Cashel K, Lewis J, Bennett S, Berzins J, Waters AM, et al. National dietary survey of adults 1983. No 3. Nutrient intakes by capital city. Canberra: Australian Government Publishing Service, 1989.
24. Watts R, Warham J. Structure of some intact lipids of petrel stomach oils. Lipids 1976;11:423–9.
25. Bishop DG, Ritz DA, Hosie GW, Kenrick JR. Fatty acid composition of the lipids of *Puffinus tenuirostris* (Temminck) in relation to its diet. J Exp Mar Bio Ecol 1983;71:17–26.
26. 1993 MIMS Annual: 17th Australian edition. Crows Nest, NSW: MIMS Australia, 1993.
27. Place AR. Comparative aspects of lipid digestion and absorption: physiological correlates of wax ester digestion. Am J Physiol 1992;263:R464–R71.
28. Skira I. Commercial harvesting of short-tailed shearwaters (Tasmanian mutton-birds). In: Meek PD, O'Brien PH, eds. Wildlife use and management: report of a workshop for Aboriginal and Torres Strait Islander people. Canberra: Australian Government Publishing Service, 1992:7–18.
29. Wilson G, McNee A, Platts P. Wild animal resources: their use by Aboriginal communities. Canberra: Australian Government Publishing Service, 1992:28: 80–1.
30. Paul AA, Southgate DAT. The composition of foods, 4th edition. London: Her Majesty's Stationery Office, 1978.

Processed foods

The ten pages of *Processed Foods*, produced by the Food Information Service, 65 Berry Street, North Sydney, NSW 2060, contain information on processing, controls on ingredient and labelling of processed foods, preservation techniques used—including dehydration, chilling, freezing, canning, pasteurisation and stabilisation with additives—and on nutritional content. This is a particularly useful summary of the topic, clearly presented. Copies are available, on request, from the above address, or Private Bag 938, North Sydney, NSW 2059.

Developments with Marine Oil Products in Australia

Peter Nichols[1,2], Michael Bakes[1], Ben Mooney[1], Nick Elliott[1] and Chris Strauss[3]
[1]CSIRO Division of Marine Research, Hobart, Tasmania, Australia; [2]Antarctic CRC, Hobart, Tasmania, Australia; [3] CSIRO Division of Molecular Science, Clayton, Victoria, Australia

FAX: 61 3 62325123 E-mail: Peter.Nichols@marine.csiro.au

SUMMARY

The waste, by-catch and by-products from the Australian fishing industry are value-added to yield a range of marine oil products. Over the past decade, research has focused on oil from deep water and pelagic fish and several companies commenced production of marine oils for export and local use. Oils of interest include: 1. wax esters derived from orange roughy, oreo dories and other fishes, 2. shark liver oils containing squalene and diacylglycerol ethers, and 3. triacylglycerol oils rich in essential omega-3 fatty acids. These oils are used as lubricants, in degreaser and hand cleaner products, in cosmetics and nutraceuticals. Our research involves characterisation of marine oils from Australian species, searching for new sources for commercially sought-after oils, development of new or refinement of existing processes suitable for Australian oils, and transfer of know-how to industry. Strong links exist with industry, giving an increased return for both the fishermen and oil processors, without increasing catch effort.

INTRODUCTION

Over the last ten years, facilities have been established for the production of oil and high grade meal from south-eastern Australian fisheries, including orange roughy, which in the early 1990s was one of Australia's larger and economically more important catches. Through Fisheries Research and Development Corporation support, CSIRO expanded the knowledge-base and technical capability on these and other marine oils. Research also draws on the expertise of industry with the objective of exploiting the by-catch and by-products of Australia's south-eastern fisheries, for example, orange roughy, jack mackerel, tuna, deep-sea shark and other species. Oil products with potential for development include: (i) oils rich in wax esters, (ii) squalene and other components from livers of deep-sea sharks and (iii) oils enriched in omega-3 [or (n-3)] polyunsaturated fatty acids (PUFA).

Research centres on characterization of marine oils from a range of species, searching for sources of various sought after oils, development of new or refinement of existing processing conditions suitable for use with Australian oils and transfer of know-how and technology to industry. Specific objectives include:

1. Development of new marine oil-based and other value added products from the by-catch and waste generated by the fishing and related industries.
2. Determine the nutritional composition of commercial and non-commercial fishes, with an emphasis on oils.
3. Undertake strategic studies in an environmental and ecological context of the oil composition of key species, including microbial, from Antarctic lakes and southern ocean waters. These data will also be used in trophodynamic and other studies.
4. Assist Government, industry and the community with advice and research on issues relevant to the development and use of marine living resources.

MATERIALS AND METHODS

Samples from raw liver, other tissue or aliquots of crude oils prepared by industry were analysed. Industrial processing of shark liver oils varies, however, it involves maceration of liver followed by heating at 50 to 60°C, standing to separate solids, and finally decantation (or centrifugation and filtration). When raw livers or other materials (e.g. flesh, algae, bacteria) were analysed, the Bligh and Dyer (1959) technique was used for extraction. Non-saponifiable lipids such as glyceryl ether diols, derived from diacylglyceryl ethers present in shark liver oil, and hydrocarbons (predominantly squalene) were isolated following saponification. Glyceryl ether diols were converted to their trimethylsilyl ethers using BSTFA. Fatty acid methyl esters (FAME) (from diacylglyceryl ethers and triacylglycerols) were liberated by saponification and subsequent methylation or formed directly by transesterification. Oils were analysed for lipid composition using a thin-layer chromatograph with flame ionisation detection. Gas chromatography was performed on non-saponifiable lipid and FAME fractions. Instrumental and other methods used in oil characterisation are detailed in Bakes and Nichols (1995).

RESULTS AND DISCUSSION

It is estimated that 100 000 tonnes p.a. of the Australian fish catch goes to waste. However, fish contain a diverse array of oils, which vary markedly between species. We are isolating valuable components for dietary supplements, pharmaceuticals, industrial and cosmetic uses. The strengths of the Australian Marine Oil industry include: 1. sustainability of raw material (some countries show resources in decline), 2. a clean and green image of the local resource, 3. closeness to Asian markets, 4. uniqueness of composition of several marine oils, 5. an increasing knowledge of marine oils and 6. development of appropriate technologies. Opportunities based on these strengths exist and the Marine Oils industry has taken several products into the international market place. Together these features provide the Australian fishing industry with the capacity to better utilise existing resources.

Wax ester oils

Research has provided biochemical data on orange roughy and the waste obtained from processing, thereby assisting industry to better utilise the orange roughy resource. Major fatty acids are: 18:1(n-9)c, 20:1(n-9)c and 22:1(n-11) in the swim bladder (a main organ for oil storage), with major fatty alcohols being: 20:1(n-9)c, 16:0, 22:1(n-11)c and 18:1(n-9)c (Bakes et al. 1995). Oil from orange roughy caught in Australian, New Zealand and British waters has a similar profile. From data on the oil composition of orange roughy and its properties, a range of wax ester-derived degreaser, hand cleaner and related industrial products have been manufactured in Australia by Beku Environmental Products Ltd. The oil from deep water oreos also contains high levels of wax esters and, it is feasible to use this oil in addition to that from orange roughy. Ongoing research examines the composition and physical properties of oil produced by industry, including further value-adding oils to a high purity wax ester; the latter working with Beku on process refinement.

Shark liver oils

Deep sea sharks are a significant by-catch of the orange roughy and other fisheries. Shark livers are large (20% of the total shark's weight) and contain considerable quantities of oil which is often enriched in squalene (Fig. 1A). Squalene is used as a health-food or is hydrogenated to squalane, which is used in the pharmaceutical and cosmetic industries as a lubricant and cosmetic base. We continue to evaluate the squalene content of deep-sea shark species landed in Australian (Bakes and Nichols 1995) and other waters, and assist industry in screening the squalene content of oils.

Research was completed on processing of crude oils so that the opportunity existed to export refined squalene, rather than crude shark liver oil. Several processes for separating and purifying

squalene have been established and at present Beku is the sole Australian manufacturer of squalene. Beku has further developed a diacylglyceryl ether (DAGE, Fig. 1B) fraction from shark liver oil. This new product is also used in nutraceuticals. From 1995 to 1997, technology has been developed for the preparation and purification of squalane within Australia. As for squalene, options for use of by-products from squalane purification are being examined.

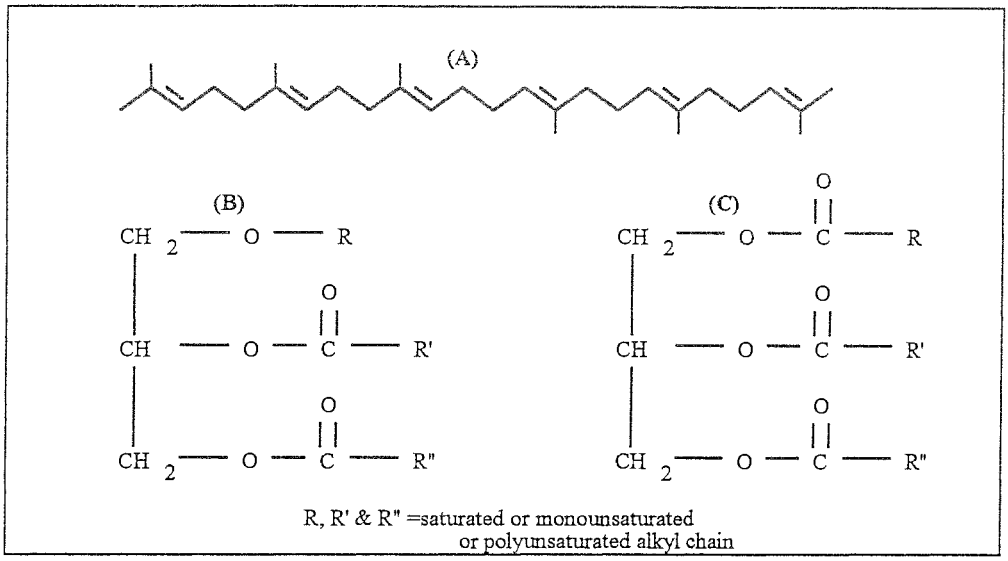


Figure 1. Structure of (A) squalene, (B) diacylglyceryl ether, and (C) triacylglycerol.

Omega-3 Polyunsaturated Fatty Acid (PUFA) oils

There is increasing scientific evidence that inclusion of the fish-derived long-chain PUFA in the human diet reduces the incidence of coronary heart disease, stroke and other disorders (e.g. dyslexia, atherosclerosis, childhood asthma). Omega-3 PUFA oils are also thought to be beneficial for brain and retina development and function. Capsules of fish oils with high levels of the essential PUFA, eicosapentaenoic acid [EPA, 20:5(n-3)] and docosahexaenoic acid [DHA, 22:6(n-3)], are marketed overseas. Imported products (e.g. MaxEPA), with around 30% EPA and DHA, have captured a small market in Australia. More recently (n-3) oils have been incorporated in other food items; for example, in Scandinavia oils are added to bread. Further processing can produce (n-3) oils containing higher levels of EPA and DHA (approx. 70-80%).

The level of EPA and DHA in oils from selected Australian fish is shown in Fig. 2. The demand for fish-derived or related (n-3) oils in Australian mariculture is increasing. By determining the oil composition of Australian species (e.g. tuna, jack mackerel) and examining ways to add value to the crude (n-3) oils, industry will be better placed to identify alternative feedstocks and to take advantage of market opportunities. Other species examined include European carp and ling.

Uses described above have encouraged the establishment of technologies in Australia for adding value to the omega-3 oils. To date a process has been developed in the laboratory for potential use in the purification of triacylglycerol-containing oils (Fig. 1C) and scale-up of the process is underway in association with Nu-Mega Lipids, a joint venture between Clover Corporation and Heinz. The Nu-Mega Lipids initiative will utilise a resource that would otherwise be disposed of as waste and this material now will used to produce refined tuna oil products. Research on enrichment technologies is underway with several products, containing in excess of 90% omega-3 PUFA, prepared in laboratory trials.

In addition to marine product research, an FRDC study was established to examine a wider range of Australia's commercial and non-commercial fishes for their nutritional composition, with an emphasis on oils. This research is due for completion in late 1997.

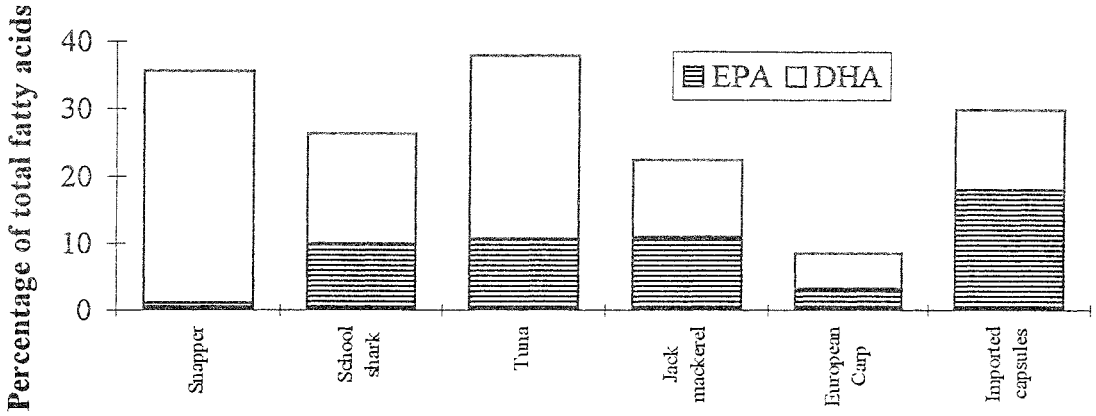


Figure 2. EPA and DHA content of oils from Australian species and imported capsules.

Antarctic Microorganisms

Lipid studies of microorganisms, zooplankton and fish from the Southern Ocean have increased understanding of the biochemical and microbial processes of the Antarctic ecosystem. Specific lipids are used in the taxonomy of new Antarctic bacteria, including those able to biosynthesise potentially useful natural products (e.g. omega-3 and omega-6 PUFA). Lipids have been used to elucidate krill, zooplankton and other food-chain interactions, including those of commercial interest. In pilot studies the effect of UV-B on microalgal lipids has been examined. Methodologies also have been transferred to aquaculture and environmental studies. For example, chemical signatures for important dimethylsulphide-producing (*Phaeocystis)* and other microalgae have been developed and used to estimate the abundance of these species, and in recent research we have demonstrated the enrichment of omega-3 PUFA into rotifers using Antarctic bacteria. Scope exists to transfer techniques and processes developed by the marine oils industry to biotechnological applications of Southern Ocean species.

The Australian Marine Oils industry has made rapid progress and opportunities are available for consolidation of developments to date and commencing new initiatives. Ongoing oil characterization and process oriented research will complement and strengthen existing industry initiatives and allow the Marine Oils industry to maximise returns on the present fish catch.

Acknowledgments

Research was supported by Fisheries Research and Development Corporation. Beku Environmental Products, Clover and Squalus and others are acknowledged. We thank C. Fandry, P. Virtue, T. Cableski and CSIRO colleagues, D. Nichols, T. Lewis, C. Mancuso, T. McMeekin and members of the Antarctic CRC Microbial Processes subprogram. Rick Phleger of San Diego State University is also thanked for support during the project.

References

Bakes, M. J. and Nichols, P. D. (1995) Lipid, fatty acid and squalene composition of liver oil from six species of deep-sea sharks collected in southern Australian waters. Comp. Biochem. Physiol. 110B: 267-275.

Bakes, M. J., Elliott, N. G., Green, G. J. and Nichols, P. (1995) Within and between species variation in lipid composition of Australian and North Atlantic oreo and orange roughy: regional specific differences. Comp. Biochem. Physiol. 111B: 633-642.

Bligh, E. G. and Dyer, W. M. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Phyiol. 35: 911-917.

Fisheries Research and Development Corporation

Peter Dundas-Smith[1] and Peter D Nichols[2,3*]

[1] Fisheries Research and Development Corporation, Deakin, ACT
[2] CSIRO Division of Marine Research and [3] Antarctic CRC, Hobart, Tasmania

* Presented by Peter Nichols on behalf of FRDC at the Inaugural meeting of the Australasian Section, American Oil Chemists Society, February 27-28, 1997, Canberra.

The Fisheries Research and Development Corporation (FRDC) is a national organisation responsible to its stakeholders for planning, funding and managing research and development programs. The FRDC also promotes the dissemination, adoption and commercialisation of results of the research and development. Further details on the Australian industry are available in the FRDC Research and Development plan (1).

While expansion of aquaculture continues, fishing remains a largely "hunting" operation - that is finding and catching fish in the wild. Recent and projected global seafood production is shown in Table 1.

Table 1. Global Seafood Production (from reference 1).

	Millions of metric tonnes of Seafood	
Year	Aquaculture	Wild
1986	12	93
1993	21	85
2000	35	85
2025	77	85

The value of Australian fisheries is dominated by high priced crustaceans and molluscs, with some 97% of Australians eating seafood. The average per capita consumption is more than 12 kilograms per year (Table 2).

Table 2. Australian Fish Production and Consumption (from reference 1).

Production (wild and aquaculture)

1992-93	$1.5B
1993-94	$1.7B
1994-95	$1.8B

(rock lobster, other fish, prawns, abalone, pearls, tuna, scallops, oysters, other crustaceans and molluscs)

Consumption (yearly, Australia)

1977	10.1 kg/person
1991	12.1 kg/person

Research Programs

Three FRDC Research Programs have been established. They are:

Resources sustainability. Goals: Develop Australia's wild fish resources in a sustainable manner. Research areas: Resources status and Fisheries management improvement.

Ecosystems Protection. Goals: Protect the Australian ecosystems upon which fisheries and aquaculture depend. Research areas: Ecosystems status, Ecosystems maintenance and improvement and Ecosystems management improvement.

Industry Development. Goals: To enhance the competitiveness and resilience of the Australian fishing industry. Research areas: Aquaculture development, Health and safety, Information delivery, Market development, People development, Quality Technology and Value-adding.

The FRDC supports marine oils research on a project by project basis. Representative FRDC projects that have examined various aspects of fish and related oils include:

- Fish oil and coronary heart disease,
- Marine oils from Australian fish: characterisation and value-added products,
- Abalone aquaculture sub-program - optimisation of essential lipids in artificial feeds for Australian abalone,
- Identification of red pigment in orange roughy, yield and separation,
- Nutritional composition of Australian seafood.

Outcomes from this research have included the development and ongoing production of various wax ester products from orange roughy oil research, and (2) abalone dietary studies highlighting the effect on growth (including seasonal) of different oils (G. Dunstan et al., personal communication). As well an increasing knowledge of the nutritional composition of Australia's commercial and non-commercial (trash) fish and other seafood has been gained (P. Virtue et al., unpublished data) as evidenced by recent media coverage (e.g. Sydney Morning Herald, February 26).

In possible future developments, the potential exists for a new Australian krill fishery based on *Euphausia superba* from the Southern Ocean. Potential krill harvest estimates are up to five to tenfold greater than world-wide total landings of finfish (P. Virtue, personal communication). As krill can be rich in oil, including the essential polyunsaturated fatty acids EPA and DHA, further research would be beneficial on this species.

References

1. Investing for Tomorrow's catch. FRDC Research and development plan, 1996-2000. ISSN 1320-3754.
2. Nichols, P. D., Nichols, D. S. and Bakes, M. J. (1994) Developments with Marine Oil Products in Australia. AOCS Int. News on Fats, Oils and Related Materials (INFORM). 5: 254-261.

Progress with Marine Oil Products in Australia

Peter Nichols[1,2], Michael Bakes[1], Ben Mooney[1], Nick Elliott[1] and Chris Strauss[3]

[1] CSIRO Division of Marine Research, Hobart, Tasmania, Australia; [2] Antarctic CRC, Hobart, Tasmania, Australia; [3] CSIRO Division of Chemicals and Polymers, Clayton, Victoria, Australia

Summary

The waste, by-catch and by-products generated by the fishing industry may be value-added through various procedures to yield a range of marine oil products. A 1930s CSIRO report suggested that the oil and vitamin content of a range of shallow and mid water Australian fish had potential to be value-added. After the second world war, the availability of synthetically produced vitamins saw the use of fish-derived oils diminish. By the 1980s, research also began to focus on oil from deep water fish and several companies commenced to produce marine oils for export and local use.

Oils of current interest include: 1. wax esters derived from orange roughy, oreo dories and other species, 2. shark liver oils containing squalene (which also can be converted to squalane) and diacylglycerol ethers, and 3. triacylglycerol oils rich in the essential omega-3 fatty acids. Several uses for the oils exist including as lubricants, in degreaser and hand cleaner products, in health and nutritional products and in cosmetics. Research conducted within CSIRO currently centers on the detailed characterization of marine oils from a range of species, searching for new sources for the commercially sought-after oils, development of new or refinement of existing processing conditions suitable for use with the Australian oils, and transfer of know-how and technology to industry. The research has established strong ties with local industry, giving an increased return for both the fishermen and oil processors, without an increase in catching effort.

Introduction

The Australian Science and Technology Council (ASTEC), in a 1988 report on post-harvest technologies and opportunities in the fishing industry, stated "fish are big business in this country, but we could get a better return on the catch". It was also revealed that at the time the level of processing of by-products from Australia fisheries was low. Most of the by-product from finfish was used in pet food, fishing bait or in the production of meal and unrefined oils. The potential existed for production and export of products of higher value from the oil and meal of Australian commercial fishes. Based on the nature of the fisheries resource, ASTEC indicated that better use of the by-catch (non-targeted species) was essential to avoid wasting the fishery resource.

A number of companies established facilities for the production of oil and high grade meal from south-eastern Australian fisheries, including for orange roughy fishery, which at the time was one of Australia's larger and economically more important catches. Through the support of the Fisheries Research and Development Board, the CSIRO Marine Products Project has developed a considerable intellectual resource and technical capability on these and other marine oils. Much of the research conducted involves close collaboration between scientists from the Divisions of Marine Research and Chemicals and Polymers. In general all marine oils and related research currently is aligned to the Marine Products and Biotechnology Component of the CSIRO Marine Sector.

Oils under research are supplied directly by industry or are obtained from fish and related samples by solvent extraction using standard procedures. The oil composition is determined using state-of-the-

art methods. Research undertaken draws together the expertise of industry and CSIRO with the objective of exploiting the by-catch and by-products of Australia's south-eastern fisheries, for example, orange roughy, jack mackerel, tuna, 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 (or n-3) polyunsaturated fatty acids (PUFA).

Research conducted by the project currently centers on the detailed characterization of marine oils from a range of species, searching for new sources for various commercially sought after oils, development of new or refinement of existing processing conditions suitable for use with the Australian oils and transfer of know-how and technology to industry. Specific objectives include:
1. Assist Australian industry in the development of new marine oil-based and other value added products from the by-catch and waste generated by the fishing and related industries.
2. Determine the nutritional, with an emphasis on oils, composition of Australia's commercial and non-commercial fishes. These data also will be used in wider trophodynamic and other studies.
3. Undertake strategic studies of (i) the oil composition of key, including microbial, species from the southern ocean environment and (ii) microbial processes within Antarctic lakes and fjords in an environmental and ecological context.
4. Assist Government, industry and the community with advice and research on issues relevant to the development of marine living resources.

The research has established strong ties with local industry, resulting in an increased return for both the fishermen, oil processors and Australia's developing marine oils industry, without an increase in catching effort. In the Marine Products Project the intellectual property, technologies and know-how developed through research and development on marine and related oils are also transferred to other scientific endeavours, including strategic trophodynamic, environmental and other studies.

Materials and Analytical Methods

Material analysed was from raw liver, other tissue or aliquots of crude oils prepared by local industry. Processing techniques used by industry in the preparation of shark liver oil vary, but involve mechanical maceration of liver, followed by heating at 50-60°C, standing to allow the solids to separate, and finally decantation (or possible centrifugation and filtration). When raw livers or other materials (e.g. flesh, algae, bacteria) were analysed, a modification of the technique developed by Bligh and Dyer (1959) was used for extractions. Oils were then concentrated using rotary evaporation. Crude oils supplied by industry were directly diluted with chloroform. Diluted oils were then analysed for lipid composition using a MK V thin-layer chromatograph with flame ionization detection (TLC-FID, Iatron Laboratories, Japan); Iatroscan results with the MK III analyser are generally reproducible to ±10% (Volkman and Nichols, 1991) and the MK V system is reproducible to ±3-5% (unpublished data).

For laboratory analyses, non-saponifiable lipids such as glyceryl ether diols, derived from the diacylglyceryl ethers present in the shark liver oil, and hydrocarbons (predominantly squalene) were isolated following saponification. Glyceryl ether diols were converted to their corresponding trimethylsilyl ethers using N,O-bis-(trimethylsilyl)-trifluoroacetamide and made up to a known volume with an internal injection standard. Fatty acid methyl esters (from diacylglyceryl ethers and triacylglycerols present in the crude liver oil) were liberated by saponification and subsequent methylation or formed directly by transesterification.

Gas chromatographic (GC) analyses of non-saponifiable lipid and fatty acid fractions were performed with an 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 methyl tricosanoate as an internal injection 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, Western Australia. Individual components were identified using mass spectral data and by comparing retention time data with those obtained for authentic and laboratory standards. GC results are subject to errors of ±5%. Gas chromatographic-mass spectrometric (GC-MS) analyses were performed on either an HP 5890 GC and a 5970 Mass Selective Detector (MSD) fitted with a direct capillary inlet and JADE-valve injector (SGE, Australia) or by a FISONS MD800 GC-MS. The GC was fitted with a capillary column similar to that described above. Other methods used in oil characterization are described in Bakes and Nichols (1995a).

Results and Discussion

It is estimated that around 100 000 tonnes per annum of the Australian fish catch would normally go to waste. However, fish contain a diverse array of oils, which vary markedly between species. We are isolating valuable components for cosmetic, dietary supplements, pharmaceuticals and industrial use.

Strengths for the Australian Marine Oil industry include: 1. the sustainability of the raw material (in comparison, several competing countries show indications that raw resources are in decline), 2. in particular the clean and green image and nature of the local resource (c.f. competitors), 3. closeness to Asian markets, 4. the uniqueness of the composition of several of Australia's marine oil resources, 5. a good technical knowledge of the composition of local marine oils and 6. the now on-line development of appropriate technologies. Opportunities based on these strengths exist and the Australian Marine Oils industry has broken into or is well placed to break into the international market place for several products. Clear opportunities for developing further new products exist, based on the uniqueness of the raw resource and the clean and green nature of Australian southern ocean oils. Together these and other features will provide the Australian fishing industry with the opportunity to improve its image through better utilization of existing and other waste resources. In the following discussion, progress on the development of Australian products from the three main types of marine oils will be presented.

Wax ester oils

One aim of our research has been to provide high quality biochemical data on orange roughy and the waste obtained from processing, thereby assisting industry to better utilize the orange roughy resource. Our early research showed that oil in orange roughy caught in Australian, New Zealand and British waters has a broadly similar distribution of chemical components. Major fatty acids were: 18:1(n-9)c, 20:1(n-9)c and 22:1(n-11) in the swim bladder (a main organ for oil storage), with major fatty alcohols being: 20:1(n-9)c, 16:0, 22:1(n-11)c and 18:1(n-9)c (Bakes et al. 1995a; Elliott et al. 1990). At the time, these results were good news for Australian companies previously importing oil from New Zealand, in that there was a local source available. From the early knowledge of the oil composition and its properties, over the past five years a range of wax ester-derived degreaser, hand cleaner and related industrial products have been manufactured in Australia by Beku Environmental Products Ltd. based in Hobart.

The oil composition of deep water oreos was examined. The oil from many oreo species also contains high levels of wax esters and based on these results, it was feasible to use this oil in addition to that derived from orange roughy. Ongoing work determines the composition and physical properties of oil derived from the variety of processes used by industry, including the further value-adding of the oil to high purity wax ester. Research on the latter involves working closely with Beku on process refinement.

Shark liver oils

Deep sea sharks are a significant by-catch of the orange roughy and other fisheries. The livers of these sharks are large (about 20% of the total shark's weight), and they contain considerable quantities of oil which is often enriched in the hydrocarbon squalene (Figure 1). Squalene is used in Asian countries as a health-food 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. Research is also underway examining the possible use of shark liver oil fractions in vetinerary animal health. There is scope to increase the amount of oil exported from Australia and in particular to refine the oil into value-added products. We are continuing to evaluate the squalene content of deep-sea shark species landed in Australian and other waters (e.g. Figure 2, see also Bakes and Nichols 1995, Bakes etal. 1995b), and to assist Australian industry in the use of appropriate instrumentation for rapid screening of squalene content of oils.

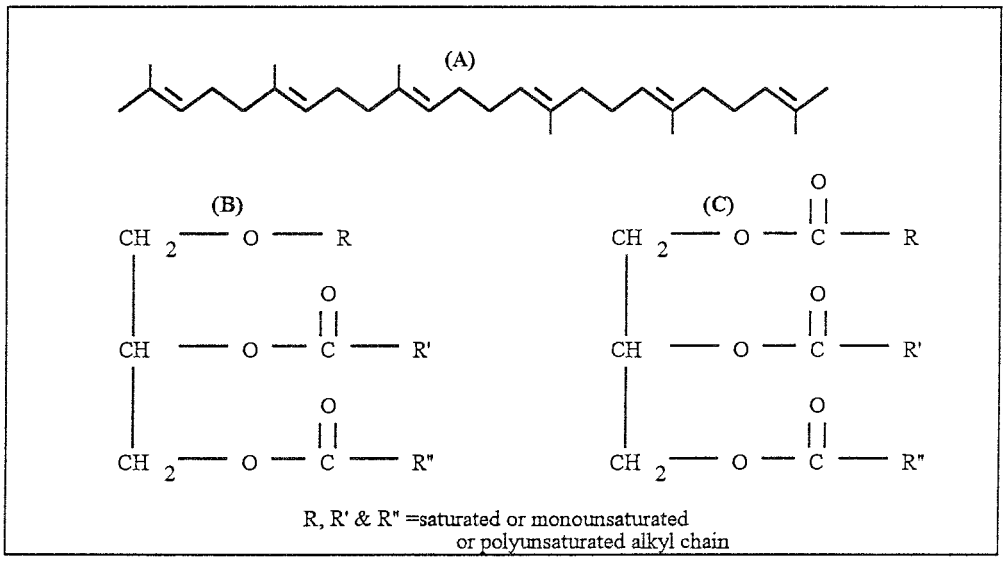


Figure 1. Structure of squalene (A), diacylglyceryl ether (B), and triacylglycerol (C).

Research has been completed on the processing of the crude oil so that the opportunity exists to export the value-added refined squalene, rather than crude shark liver oil. Several processes for the separation and purification of squalene have been established over the past 5 years and at present Beku is the sole Australian manufacturer of squalene for the local and export market. Beku has very recently further developed their operation to manufacture a diacylglyceryl ether (DAGE, Figure 1) fraction from shark liver oil. This new product is also for use in nutraceuticals. High levels of DAGE have recently been discovered in a lower member of the southern ocean food-chain (Phleger et al., unpublished data). Further research is required to determine whether the potential exists to culture such an organism as an alternate and possibly renewable source of DAGE.

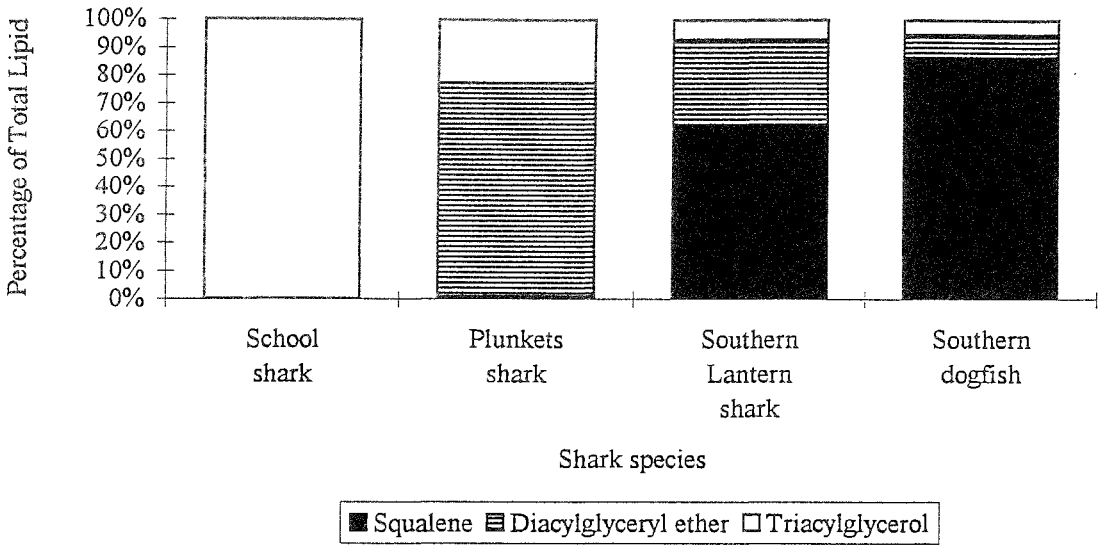


Figure 2. Lipid class composition (determined by TLC-FID) of liver oils from selected Australian deep-sea and other (school) shark species.

From 1995-1997, the Divisions of Marine Research and Chemicals and Polymers have worked together towards the development of technology for the preparation and purification of squalane within Australia (Figure 3). As for the squalene process, options for uses of by-products from squalane purification currently are being examined.

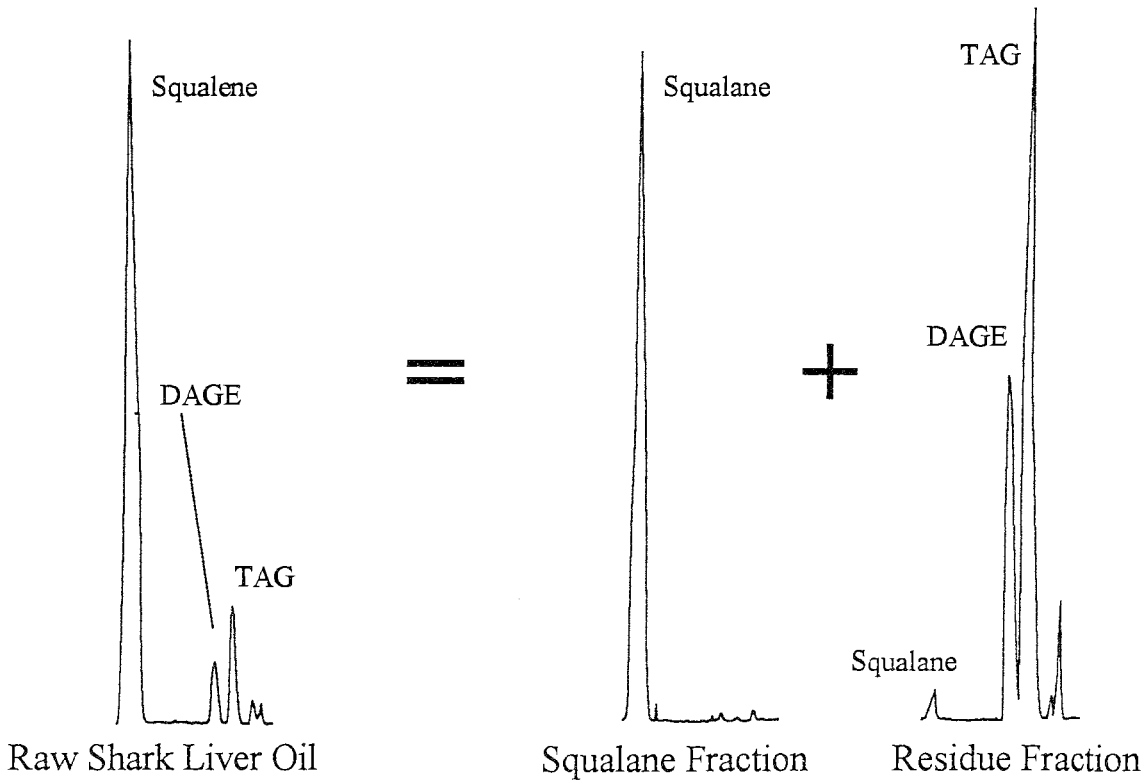


Figure 3. TLC-FID traces illustrating lipid class composition of raw shark liver oil and separated products after processing to produce squalane. Abbreviations: TAG, triacylglycerol; DAGE, diacylglyceryl ether.

Omega-3 Polyunsaturated Fatty Acid (PUFA) oils

There is increasing scientific evidence that inclusion of the long-chain fish-derived PUFA in the human diet can reduce the incidence of coronary heart disease, stroke and other disorders (e.g. dyslexia, atherosclerosis, childhood asthma). The omega-3 PUFA oils are also thought to be beneficial for brain and retina development and function. Capsules of fish oils containing high levels of the essential PUFA, eicosapentaenoic acid [EPA, 20:5(n-3)] and docosahexaenoic acid [DHA, 22:6(n-3)], are aggressively marketed overseas, and imported products (e.g. MaxEPA), containing around 30% EPA and DHA, have captured a small market in Australia. More recently (n-3) oils have been incorporated in other food items; for example, in Scandinavia the oils are now added to bread. The second 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-derived or related (n-3) oils by the Australian mariculture industry will also 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, which contains 25-30% EPA plus DHA. The fishery failed in 1989, and oils were imported at considerable cost; data were not available on possible local replacements. By determining the oil composition of Australian species (e.g. tuna, jack mackerel, see Figure 4) and examining ways to add value to the crude (n-3) oils, we will be better placed to identify alternative feedstocks and to take advantage of new market opportunities in other industries. Other species being examined include European carp and ling.

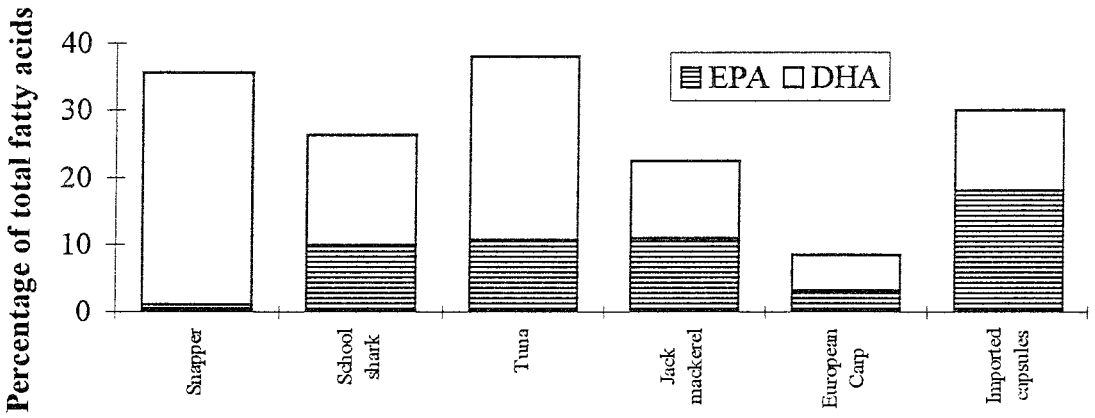


Figure 4. EPA and DHA composition (determined by capillary GC) of oils from Australian species and imported fish oil capsules.

The potential uses described above have resulted in the establishment of commercial interest and technologies in Australia for adding value to the omega-3 oils. To date a process has been developed in the laboratory for potential use in the purifying of these oils (Figure 5) and work is underway to scale-up the process in association with Nu-Mega Lipids, a recently established joint venture between Clover Corporation and Heinz. The Nu-Mega Lipids initiative will utilize a product that would be otherwise disposed of as waste and is expected to be a leading producer of refined tuna oil.

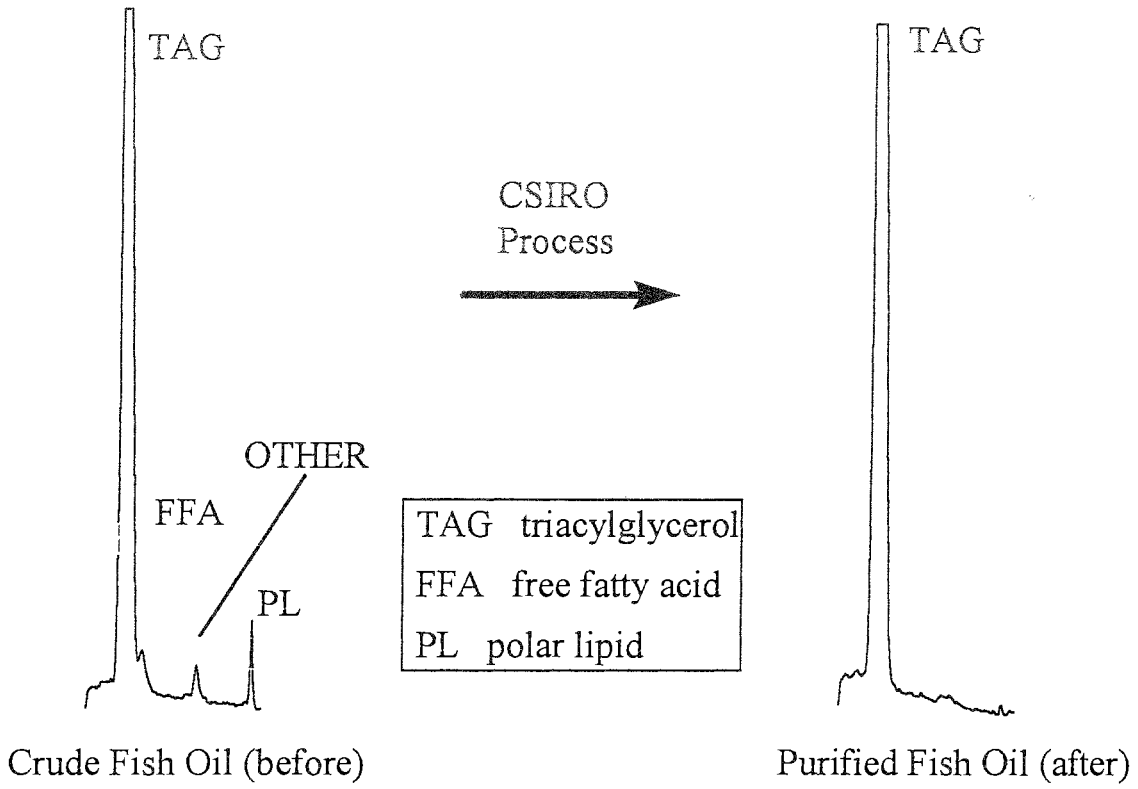


Figure 5. TLC-FID traces illustrating lipid class composition of crude tuna oil and purified product.

Further research and development on enrichment technologies is presently underway with several potential products, containing in excess of 90% omega-3 PUFA, having been prepared in laboratory trials. It is foreseen that these technologies also will be transferable to microbial-derived oils, including oils derived from algae, bacteria and other microorganisms.

In addition to research directed to adding value to marine oils derived from the waste and by-catch, a new proposal was funded by the FRDC to examine a wider range of Australia's commercial and non-commercial fishes for their nutritional, with an emphasis on oils, composition. The emerging national Marine Oils industry also has expressed interest in the outcomes of this research which is due to be completed in late 1997.

Antarctic Microbes

In related strategic studies in the Southern Ocean Environment, the broader approaches of the Marine Products Project are applied to understanding the biochemistry and role of key Antarctic species and microbial processes. Antarctic lakes are model systems for the complex interactions found in coastal and open ocean waters. Chemical signatures for important dimethylsulphide-producing (*Phaeocystis)* and other microalgae have been developed and used to estimate the abundance of these algae in Southern Ocean waters. Specific lipids are used to aid the taxonomy of new Antarctic bacteria, including those of commercial interest based on their ability to biosynthesise potentially useful natural products (e.g. omega-3 and omega-6 PUFA), and to elucidate krill-zooplankton food-chains. Pilot studies on the effect of UV-B on algal lipids also have been performed.

The techniques the group has developed and routinely uses are transferred to wider trophodynamic, including aquaculture, and environmental studies. For example, a recent highlight has been the successful enrichment of omega-3 PUFA into rotifers using Antarctic bacteria. This research is to be expanded as part of a PhD project to commence in 1997. Similarly, the oil content and composition of other key species, including those of commercial interest from the southern ocean, is examined as part of several food-web studies. This research involves collaboration with scientists from overseas institutions. The Antarctic studies performed are part of the CSIRO commitment to the Antarctic CRC with much of the research undertaken by postgraduate students.

In conclusion, the Australian Marine Oils industry has made rapid progress. The opportunities are available now for consolidation of research and development to date and the commencement of new initiatives. The industry also will need further research and development. Ongoing characterization and process oriented research will complement and strengthen existing industry initiatives and will allow the Marine Oils and kindred industries to maximize the return for the Australian fishing industry.

Acknowledgments

Research has been and continues to be supported by the Fisheries Research and Development Corporation (91/77, 94/115). The ongoing interest and support by Beku Environmental Products, Clover and Squalus and others is gratefully acknowledged. We thank Chris Fandry, Ben Mooney, Patti Virtue, Danny Holdsworth, Teresa Cablewski, Greg Simpson and CSIRO colleagues, together with David Nichols, Carol Mancuso, Tom McMeekin and members of the Antarctic CRC Microbial Processes group and Rick Phleger of San Diego State University for support during the project.

References

Bakes, M. J. and Nichols, P. D. (1995) Lipid, fatty acid and squalene composition of liver oil from six species of deep-sea sharks collected in southern Australian waters. Comp. Biochem. Physiol. 110B: 267-275).

Bakes, M. J., Elliott, N. G., Green, G. J. and Nichols, P. (1995a) Within and between species variation in lipid composition of Australian and North Atlantic oreo and orange roughy: regional specific differences. Comp. Biochem. Physiol. 111B: 633-642.

Bakes, M. J., Nichols, P. D. and Strauss, C. R. (1995b) Value-added products from Australian shark liver oils. In: Recent Advances in Marine Science and Technology. Proceedings PACON 1994 Conference (O. Bellwood, H. Choat and N. Saxena, Eds.) pp 47-53, Townsville, July 1994, James Cook University of North Queensland.

Bligh, E. G. and Dyer, W. M. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., 35: 911-917.

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

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.

DOCOSAHEXAENOIC ACID-RICH LIVER OILS FROM TEMPERATE AUSTRALIAN SHARKS

Peter D. Nichols, Michael J. Bakes and Nicholas G. Elliott

CSIRO Division of Marine Research, GPO Box 1538, Hobart, Tasmania 7001 Australia

Proposed running title: PUFA-containing liver oils from temperate Australian sharks

keywords: *Mustelus antarcticus, Galeorhinus galeus*, *Squalus acanthias*, lipids, triacylglycerol, fatty acids

mailing address Peter D Nichols, CSIRO Division of Marine Research,
GPO BOX 1538, Hobart 7001, Tasmania, Australia.
Phone: +61 3 6232 5222
Fax: +61 3 6232 5000

23 **ABSTRACT**

24 The livers from the two main commercially-targeted shark species in southern Australia (*Mustelus*
25 *antarcticus*, gummy shark and *Galeorhinus galeus*, school shark), together with *Squalus*
26 *acanthias* (white-spotted spurdog), were analysed for oil content and composition and fatty acid
27 composition. The yield of oil from the liver was 30 to 64% (wet weight) for *M. antarcticus* and 50
28 to 53% (wet weight) for *G. galeus*. Lipid classes were determined by thin-layer chromatography
29 with flame ionisation detection (TLC-FID), with the major lipid being triacylglycerol (≥95%);
30 other minors lipids were polar lipid, wax ester, sterol (mainly cholesterol) and free fatty acid.
31 Long-chain omega-3 polyunsaturated fatty acids (PUFA) accounted for between 33 and 39% of the
32 total fatty acids in all three species and docosahexaenoic acid (DHA) levels were between 13 and
33 18%. The liver oils of *M. antarcticus* and *G. galeus* and other shark species may be an attractive
34 source of omega-3 fatty acids, specifically DHA, for direct use and/or for adding further value.
35

INTRODUCTION

The main sources of EPA and DHA-containing oils overseas have included pilchard, sardine, menhaden, cod, jack mackerel and tuna. In Australia the main species landed, from which oil is extracted, are jack mackerel and tuna. Overseas, the long term availability of various species as sources of oil is not guaranteed due to decreased catches. Consequently, it is desirable to identify new sources of oils rich in EPA and DHA that are easily accessed.

The southern Australian shark fishery currently has an annual production of about 3 500 t and is worth in excess of $15 million to the fishermen (or in excess of $50 million, retail value) (Campbell et al. 1992). The fillets, in some regions of Australia referred to as "flake", is marketed for human consumption and is mainly taken from gummy (*Mustelus antarcticus* Günther 1870) and school (*Galeorhinus galeus* Linnaeus 1758) sharks (Last and Stevens 1994). The production of fillets usually results in a large quantity of by-product and waste, both of which currently have little commercial value. Value adding of by-products has been successful in Australia for other shark species (eg. deep-sea dogfish as a source of purified squalene and diacylglyceryl ethers) (Nichols et al. 1994; Bakes and Nichols 1995).

Although *M. antarcticus* (gummy shark) and *G. galeus* (school shark) are the targeted species in southern Australia for their flesh, *Dalatias licha* (black shark) and other species such as *Squalus acanthias* (white-Spotted Spurdog) are occasionally substituted. The sharks of interest are generally between 0.8 and 1.6 m in length and weigh between 2 and 25 kg (John Stevens, personal communication). The waste generated by this fishery is in excess of 1650 t per annum and includes skin, guts, head and liver, representing between 45 to 50% of the total body weight. The liver of these species usually accounts for 10% of the total body weight and based on a 50% yield

of oil from the liver, the fishery would yield approximately 150 to 200 t of liver oil from the current catch levels.

The essential omega-3 fatty acids, eicosapentaenoic acid [EPA; 20:5(n-3)] and docosahexaenoic acid [DHA; 22:6(n-3)], have been demonstrated to be beneficial in the prevention of coronary heart disease (CHD) as well as having other broader medical applications such as anti-cancer agents, infant nutrition and as feed additives (e.g. Burns and Spector 1994; Carson 1995; Kinsella 1986, and references therein). The interest in using PUFA in cancer therapy is to complement chemotherapy rather than as a dietary supplement alone. Similarly, it appears that eating fish two or three times per week results in measurable benefits with respect to parameters examined in CHD studies. There is also growing interest in PUFA addition to aquaculture feeds (Nichols et al. 1996).

The literature available on the lipids and fatty acids of the common shark species *Mustelus antarcticus* and *Galeorhinus galeus* is restricted to one study (Craig 1978) that presents lipid composition data. We present the lipid content and composition and the fatty acid profiles of the liver oils from *Mustelus antarcticus* and *Galeorhinus galeus* and *Squalus acanthias* which is currently supplemented for *M. antarcticus* and *G. galeus*.

MATERIALS AND METHODS

Sample collection. Three specimens of *Mustelus antarcticus* and *Galeorhinus galeus* were collected for their livers. *M. antarcticus* (gummy shark) specimens were from South Australian waters (total length, TL #288, 1020 mm and #285, 1200 mm; January 1994) and New South Wales (TL #165, 580 mm; June 1993), were all female and were caught at ca. 90 m depth. For *G.*

84 *galeus* (school shark), two specimens (Total length, TL; #127, 805 mm and #128, 560 mm; June
85 1994) were from Tasmanian waters and the other (TL #27, 960 mm; February 1994) from New
86 Zealand; all samples were female (reproductive status not known), catch depth data was not
87 available. All samples were stored at -20°C while in transit and then at -80°C in the laboratory.
88
89 A local oil processor in Tasmania supplied samples of liver oil from school, gummy and white-
90 spotted spurdog sharks. These oils were obtained using mechanical maceration of the livers,
91 followed by heating at 50 to 60°C, then separation for 2 to 4 days and decantation of the oil.
92
93 *Lipid extraction*. Aliquots of liver (1 to 3g) were homogenised in a pre-rinsed mortar and pestle
94 and were quantitatively transferred to a separating funnel using chloroform, methanol and water
95 (ratio 1:2:0.8 v/v/v) (Bligh and Dyer 1959). Samples were mixed and left to extract overnight.
96 Phases were separated by adding chloroform and water so that the final ratio of solvents was
97 1:1:0.9 v/v/v. Following isolation and concentration of the lower chloroform layer, samples were
98 transferred to vials and the excess solvent removed under nitrogen prior to determining the oil
99 content of the liver.
100
101 *Lipid analyses*. An aliquot of the oil (either obtained by extraction or as the crude product) was
102 diluted and analysed for lipid classes using an Iatroscan MK V TLC-FID analyser (Iatron
103 Laboratories, Japan). Two solvent systems were used to identify lipids: hexane/diethyl ether/acetic
104 acid (60:17:0.2 v/v/v) and hexane/diethyl ether (96:4) provided resolution between most common
105 lipid classes (Volkman and Nichols 1991) and reproducibility was found to be ±5% (unpublished
106 data). Data were acquired using DAPA software (Kalamunda, Australia) on an IBM-compatible
107 personal computer.

FAME analyses. An aliquot of the total lipid extract was methylated under nitrogen using a solution of methanol, chloroform and hydrochloric acid (10:1:1 v/v/v) and the fatty acid methyl esters (FAME) extracted using hexane: chloroform (4:1 v/v). FAME were then concentrated under nitrogen and treated with N,O-bis-(trimethylsilyl) trifluoroacetamide (BSTFA) to convert free hydroxyl groups to their corresponding trimethyl silyl (TMS) ethers. Samples were then blown to dryness under nitrogen and made up to a known volume in chloroform containing methyl tricosanoate (internal-injection standard). FAME were analysed using an HP 7673 autosampler and an HP 5890A gas chromatograph fitted with a split/splitless injector, an FID and an HP-1 cross-linked methyl silicone fused silica capillary column (50m x 0.32 mm id.). Hydrogen was the carrier gas. The oven temperature of 50°C was held for 1 minute before being raised to 150°C at 30°C/min, then to 250°C at 2°C/min and finally to 300°C at 5°C/min. Peaks were quantified with DAPA chromatography software. Individual components were identified by comparing retention time data and mass spectral data (Fisons MD-800 system configured similar to above) with data obtained for authentic and laboratory standards.

Vitamin A and E analyses. Representative aliquots of the oils were sent to the Australian Government Analytical Laboratories (AGAL, South Australia) for vitamin assay. Standard analytical techniques were used for determination of vitamin A and E (Brubacker et al. 1985; De Leenheer et al. 1985)

RESULTS AND DISCUSSION

Oil composition

The lipid content and composition of the liver oils from the gummy and school sharks (Table 1) was similar with both sharks containing 95 to 99% triacylglycerol. The oils also contained low levels of polar lipid (1 to 4%), wax ester (trace to 2%) and sterol (identified by gas chromatography as cholesterol; tr to 1%). Commercially produced bulk oil for the two species was similar in composition. Only trace levels (less than 0.2% of the total lipid) of free fatty acids were detected in two samples (Table 1). It is common for oils produced by industry to contain elevated levels of free fatty acid (up to 5% free fatty acid, unpublished data) due in part to poor storage, handling and/or processing conditions.

Triacylglycerols are the preferred form for storage and delivery of PUFA in health and pharmaceutical products due to their thermal and oxidative stability. The high levels of triacylglycerol in the livers from the species examined in this study is a feature of the oil that may allow rapid and efficient processing compared to oils derived from other species. In contrast, other oils currently available in Australia (eg. jack mackerel and tuna) may contain high levels of polar lipid, free fatty acid, cholesterol and other unwanted compounds (unpublished data).

Gummy shark livers showed the greatest variation in lipid content with levels ranging between 30 and 64% lipid (wet mass basis). The lipid content of the school shark livers was considerably more constant at 50 to 53%. The variation in liver oil content observed for gummy shark may be due to a range of factors such as season, sex, size and location as well as other factors.

156 Compared with livers from deep-sea sharks, the yield of oil from the liver is lower (50% oil in
157 livers from school and gummy sharks versus 80% oil in livers from deep-sea sharks), but the
158 proportion of the desired component (99% triacylglycerol versus 60 to 85% squalene) is much
159 greater. Deep-sea sharks are presently fished in Australia for their livers which yield the
160 isoprenoid hydrocarbon squalene, a high boiling point liquid used in the lubrication and cosmetic
161 industries and in nutritional products. Triacylglycerols are mainly used as a stable form of lipid to
162 provide PUFA to humans and/or in aquaculture feeds. Comparable yields of oil suggest that it
163 could be economically feasible to extract the oil from the gummy and school sharks. For example,
164 a deep-sea shark liver contains 80% oil with a range of 40 to 80% (mean 60%) of the oil being
165 squalene; the liver would contain 48% squalene, mass basis. For the gummy and school sharks
166 (liver contains approximately 50% oil), the triacylglycerol-containing liver oil would be also
167 around 48% (mass basis) suggesting that it may be feasible to value-add these oils.
168
169 *Vitamins*
170 Vitamin A (retinol) was assayed in gummy and school sharks at 6.9 and 14 mg/100g oil while
171 vitamin E (alpha-tocopherol) was assayed to be 18 and 8.3 mg/100g respectively (Table 1).
172 Literature available on the vitamin content and composition of these Australian species is limited,
173 however, the vitamin content recorded for gummy and school sharks are low compared to other
174 species sourced for vitamins. For example, the white-spotted spurdog contained 25 mg/100g of
175 vitamin E (Sunarya et al. 1996).
176
177 *Fatty acid composition*
178 The major liver oil fatty acids for both gummy and school sharks included 16:0, 22:6(n-3), 18:1(n-
179 9)c and 20:5(n-3) while minor components were 16:1(n-7)c, 20:4(n-6), 18:0 and 18:1(n-7)c

(Tables 2 & 3). EPA accounted for between 6 and 14% total fatty acid (TFA) in gummy sharks and 8 to 11% in school sharks. DHA was present at higher levels in both species (13 to 18% TFA in gummy sharks and 14 to 19% in school sharks). Within-species variation was less apparent in the school sharks than observed for gummy sharks which showed, for example, greater differences in the levels of EPA and DHA. Specimen #165 contained higher levels of EPA (14% versus 7% in the other specimens) and lower levels of DHA (13% versus 18% in the other specimens). This observation may be due to the difference in geographical location and/or other factors, as noted earlier for oil content.

The liver oil from white-spotted spurdog differed in fatty acid composition relative to the gummy and school sharks. The oils from this species contained higher levels of monounsaturated fatty acids (50% of total fatty acids), comprising 18:1, 20:1 and 22:1, with 16:0 and DHA also major components (Table 2). Total PUFA was lower in the white-spotted spurdog species compared to the gummy and school sharks, with only slightly lower levels of the two essential PUFA observed (EPA 2-4%, DHA 11-15%; Tables 2 and 3). This species currently supplements the flake market, the quantities of liver available to fish waste processors may not be as high as gummy and school shark. However, based on the similar levels of the two essential PUFA, EPA and DHA, liver oil from the white-spotted spurdog may be blended with liver oil from gummy and school sharks for applications in the preparation of feeds for PUFA enrichment.

Commercial considerations

The increased demand for oils rich in EPA and DHA has led to an expansion of the biotechnology industry, particularly the mass culture of yeasts, microalgae and bacteria. The microorganisms can be grown heterotrophically to produce PUFA-containing oils; this feature is advantageous when

204 considering the long term sustainability of some of the wild fisheries and palatability of fish oils.
205 However, the cost of production of PUFA-containing oil from these various single cell organisms is
206 considerably higher than for production of fish oils. Other issues with single cell oils include
207 potential production of undesirable components including possible toxic compounds and also other
208 unwanted lipid classes.
209
210 Our results suggest that the waste generated from existing Australian shark fisheries could be
211 better exploited with respect to potential sources of omega-3 PUFA-containing oils. Fish oils
212 currently used in Australia (e.g. MaxEPA) are largely imported and have an EPA:DHA ratio of
213 approximately 1.5. In contrast the Australian oils, such as observed in this study, show a ratio of
214 EPA:DHA of less than 1, typically around 0.6 (Table 3, and unpublished data). The increased
215 nutraitional and medical interest in DHA containing oils relative to oils where EPA > DHA may
216 see an increase in potential and ultimately demand for DHA-containing fish oils.
217
218 The levels of DHA found in gummy, school and white-spotted spurdog shark liver oils are as high
219 as some of the oils currently marketed as sources of this fatty acid (Table 3). Because the oil from
220 the gummy and school sharks is currently discarded through the disposal of the livers, the
221 opportunity exists to better exploit the waste from the fishery. Even if the current catch levels of
222 gummy and school sharks are not sustained, the fishery is likely to continue in some form in
223 southern Australia. By enhancing conversion of the waste material into value-added products, it
224 may be possible to help maximise economic yields to the fishery.
225
226 **Acknowledgment**
227 The authors wish to acknowledge the contribution of John Stevens and Terry Walker for biological
228 and market information and Stephanie Davenport and Patti Virtue for comments on the draft

229 manuscript. Fisheries Research and Development Corporation (FRDC 94/115) funded this study
230 and the interest by Australian industry (Beku and Scales) is also acknowledged.

231 **References**

232 Bakes M.J. and Nichols, P.D. (1995). Lipid, fatty acid and squalene composition of liver oil from
233 six species of deep sea sharks collected in southern Australian waters. *Comparative Biochemistry*
234 *and Physiology* **110B**(1), 267-275.
235
236 Bligh E.G. and Dyer W.J. (1959) A rapid method of total lipid extraction and purification. *Can. J.*
237 *Biochem. Physiol.* **37**, 911-917.
238
239 Brubacker G., Muller-Mulot W. and Southgate D.A.T. (1985) *Methods for the determination of*
240 *vitamins in food*, Elsevier Applied Science Publishers Ltd.
241
242 Burns C.P. and Spector A.A. (1994) Biochemical effects of lipids on cancer therapy, *J. Nutr.*
243 *Biochem.* **5**, 114-123.
244
245 Campbell D., Battaglene T. and Pascoe S. (1992) Management options for the southern shark
246 fishery, *Australian Fisheries* **2**, 7-10.
247
248 Carson S.E. (1995) The role of PUFA in infant nutrition, *INFORM* **6**(8), 940-946.
249
250 Craig J.C.A. (1978) The lipids of six species of shark, *J. Mar. Biol. Ass. U.K.* **58**, 913-921.
251
252 De Leenheer A.P., Lambert W.E. and De Ruyter M.G.M. (1985) *Modern chromatographic*
253 *analysis of the vitamins*, Marcel Dekker Inc.
254

255 Gunther A. (1870) Catalogue of the fishes in the British museum. Vol 8. Catalogue of the
256 Physostomi containing the families Gymnotidae, Symbranchidae, Muraenidue, Pegasidae and of
257 the Lophobranchii, Plectognathi, Dipnoi, Ganoidei, Chondropterygii, Cyclostomata, Leptocardii in
258 the collection of the British Museum, London, 549pp.
259
260 Kinsella J.E. (1986) Food components with potential therapeutic benefits: the n-3 polyunsaturated
261 fatty acids of fish oils, *Food Technology* Feb; 89-97
262
263 Kinsella J.E. (1987) *Seafoods and fish oils in human health and disease*, Marcel Dekker Inc.,
264 New York. 317pp.
265
266 Last P.R. and Stevens J.D. (1994) *Sharks and rays of Australia*, CSIRO Press, Australia 513pp.
267
268 Linnaeus C. (1758) Systema Naturae. 10th Edn. Tome 1, 824pp. Nantes & Pisces: 220-338.
269
270 Nichols D.S., Hart P., Nichols P.D. and McMeekin T.A. (1996) Enrichment of the rotifer
271 *Brachionus plicatilis* fed on Antarctic bacterium containing polyunsaturated fatty acids.
272 *Aquaculture* 147, 115-125.
273
274 Nichols P.D., Nichols D.S. and Bakes M.J. (1994) Recent developments in marine oil products in
275 Australia. *INFORM* (International News on Fats, Oils and Related Materials). 5(3), 254-261.
276

277 Sunarya, Hole M. and Taylor K.D.A. (1996). Methods of extraction composition and stability of
278 vitamin A and other components in dogfish (*Squalus acanthias*) liver oil, *Food Chemistry* 55(3),
279 215-220.
280
281 Volkman J.K. and Nichols P.D. (1991) Applications of thin layer chromatography-flame ionisation
282 detection to the analysis of lipids and pollutants in marine and environmental samples. *J. Planar*
283 *Chrom.* **4**, 19-26.

230 **References**

231 Bakes M.J. and Nichols, P.D. (1995). Lipid, fatty acid and squalene composition of liver oil from
232 six species of deep sea sharks collected in southern Australian waters. *Comparative Biochemistry*
233 *and Physiology* 110B(1), 267-275.
234
235 Bligh E.G. and Dyer W.J. (1959) A rapid method of total lipid extraction and purification. *Can. J.*
236 *Biochem. Physiol.* **37**, 911-917.
237
238 Brubacker G., Muller-Mulot W. and Southgate D.A.T. (1985) *Methods for the determination of*
239 *vitamins in food*, Elsevier Applied Science Publishers Ltd.
240
241 Burns C.P. and Spector A.A. (1994) Biochemical effects of lipids on cancer therapy, *J. Nutr.*
242 *Biochem.* **5**, 114-123.
243
244 Campbell D., Battaglene T. and Pascoe S. (1992) Management options for the southern shark
245 fishery, *Australian Fisheries* **2**, 7-10.
246
247 Carson S.E. (1995) The role of PUFA in infant nutrition, *INFORM* **6**(8), 940-946.
248
249 Craig J.C.A. (1978) The lipids of six species of shark, *J. Mar. Biol. Ass. U.K.* **58**, 913-921.
250
251 De Leenheer A.P., Lambert W.E. and De Ruyter M.G.M. (1985) *Modern chromatographic*
252 *analysis of the vitamins*, Marcel Dekker Inc.
253

254 Gunther A. (1870) Catalogue of the fishes in the British museum. Vol 8. Catalogue of the
255 Physostomi containing the families Gymnotidae, Symbranchidae, Muraenidue, Pegasidae and of
256 the Lophobranchii, Plectognathi, Dipnoi, Ganoidei, Chondropterygii, Cyclostomata, Leptocardii in
257 the collection of the British Museum, London, 549pp.
258
259 Kinsella J.E. (1986) Food components with potential therapeutic benefits: the n-3 polyunsaturated
260 fatty acids of fish oils, *Food Technology* Feb; 89-97
261
262 Kinsella J.E. (1987) *Seafoods and fish oils in human health and disease*, Marcel Dekker Inc.,
263 New York. 317pp.
264
265 Last P.R. and Stevens J.D. (1994) *Sharks and rays of Australia*, CSIRO Press, Australia 513pp.
266
267 Linnaeus C. (1758) Systema Naturae. 10th Edn. Tome 1, 824pp. Nantes & Pisces: 220-338.
268
269 Nichols D.S., Hart P., Nichols P.D. and McMeekin T.A. (1996) Enrichment of the rotifer
270 *Brachionus plicatilis* fed on Antarctic bacterium containing polyunsaturated fatty acids.
271 *Aquaculture* 147, 115-125.
272
273 Nichols P.D., Nichols D.S. and Bakes M.J. (1994) Recent developments in marine oil products in
274 Australia. *INFORM* (International News on Fats, Oils and Related Materials). 5(3), 254-261.
275

276 Sunarya, Hole M. and Taylor K.D.A. (1996). Methods of extraction composition and stability of
277 vitamin A and other components in dogfish (*Squalus acanthias*) liver oil, *Food Chemistry* 55(3),
278 215-220.
279
280 Volkman J.K. and Nichols P.D. (1991) Applications of thin layer chromatography-flame ionisation
281 detection to the analysis of lipids and pollutants in marine and environmental samples. *J. Planar*
282 *Chrom.* **4**, 19-26.

Table 1. Lipid class composition and vitamin content of selected liver oils isolated from school and gummy sharks.

		Percentage Composition						Oil recovery	Retinol (A)	a-tocopherol (E)
SAMPLE	**Sex/Catch location**	**WE**	**TAG**	**FFA**	**ST**	**PL**	**TOTAL**	**from liver (%)**	**mg/100g**	**mg/100g**
Gummy shark										
GS #165	F / N.S.W.	tr	95	tr	1	4	100	30		
GS #285	F / S. Aust.	2	97	-	tr	1	100	64	6.9	18
GS #288	F / S. Aust.	1	96	-	tr	3	100	47		
Commercial	unknown / Tas.	3	97	-	tr	tr	100	-		
School shark										
Sch #27	F / New Zealand	tr	98	-	tr	2	100	50		
Sch #127	F / Tas.	tr	99	-	tr	1	100	52	14	8.3
Sch #128	F / Tas.	2	96	tr	tr	2	100	53		
Commercial	unknown / Tas.	3	97	-	tr	tr	100	-		

F, Female; WE = Wax ester, TAG = Triacylglycerol, FFA = Free fatty acid, ST = Sterol, PL = Polar lipid.
tr = less than 1%; - not detected.
Commercial refers to the bulk oil produced by local Tasmanian processors.

Table 2. Fatty acid compositions of gummy and school shark liver oils (both commercial and extracted) and commercial oils from white-spotted spurdog and black shark livers.

Fatty acid	*Percentage composition*								
	Gummy shark				School shark				White-spotted
	#165	#285	#288	Commercial	#27	#127	#128	Commercial	Spurdog
14:0	1.6	2.8	2.4	2.1	3.1	3.2	2.5	3.8	3.4
15:0	0.8	0.7	1	0.5	0.6	0.7	0.8	0.5	0.6
16:0	18.5	20	20.6	18.2	16.7	19.1	17.9	14.7	16.3
17:0	1.7	1.5	1.7	0.9	0.6	0.9	1	0.5	-
18:0	8	8.4	7.9	4.9	5.5	5.7	6.1	3.9	3.2
20:0	0.3	tr	0.2	0.1	tr	0.2	0.2	0.1	tr
22:0	tr	tr	tr	tr	-	tr	tr	tr	-
Sum Saturates	**31**	**33.6**	**33.9**	**26.7**	**26.6**	**29.8**	**28.4**	**23.5**	**23.5**
i15:0	0.2	tr	0.2	0.1	0.2	0.2	0.2	0.2	0.2
a15:0	-	tr	tr	tr	tr	tr	tr	tr	tr
i16:0	0.2	tr	0.2	0.1	0.2	0.2	0.3	0.1	-
br 17:1	0.3	0.4	0.7	0.4	0.3	0.4	0.6	0.3	0.5
i17:0	0.8	0.5	0.8	0.5	0.5	0.6	0.6	0.3	0.3
a17:0	1	0.9	1	0.9	0.7	1.1	1	0.6	0.6
Sum Branched	**2.5**	**2**	**2.9**	**2**	**1.8**	**2.4**	**2.7**	**1.5**	**1.6**
14:1	-	-	-	0.1	-	tr	-	0.1	-
16:1(n-9)c	0.3	0.5	0.5	0.5	0.3	0.5	0.3	0.3	0.2
16:1(n-7)c	4.7	4.7	4.3	6.6	4.8	6.9	5.2	4.5	3.7
16:1	0.3	0.3	0.2	0.1	0.2	0.4	0.2	0.2	0.2
16:1	0.2	tr	0.2	-	0.2	0.2	0.2	-	-
18:1(n-9)c	12.1	13.9	12.4	17	17.2	18.1	12.1	18.9	20.8
18:1(n-7)c	6.6	5.3	6.1	3.9	5.4	5.1	5.2	3.5	4.3
18:1(n-5)c	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.3
20:1	3.1	3.5	2.4	5	4.5	2.7	3	6.8	14
22:1	tr	0.4	tr	1.1	1	0.5	0.9	2	6.2
Sum Monounsaturates	**27.9**	**28.9**	**26.5**	**34.5**	**33.9**	**34.7**	**27.6**	**36.6**	**49.7**
C16 PUFA	tr	-	tr	0.2	0.4	tr	tr	0.5	0.2
18:2(n-6)	1.4	1.2	1.8	0.9	1.7	1.4	1.3	1.6	1.4
18:3(n-3)	tr	tr	0.3	0.3	0.4	0.4	0.2	0.2	0.4
18:4(n-3)	0.4	0.3	0.7	0.5	1.5	0.3	0.6	1.7	-
18:3(n-6)	tr	tr	tr	-	tr	tr	tr	-	1
20:2(n-6)	1.3	0.9	1	-	0.4	0.4	0.4	-	0.3
20:3(n-6)	0.2	tr	0.2	-	tr	0.2	0.3	-	0.1
20:4(n-3)	0.5	0.4	0.5	0.6	1.3	0.5	0.6	1.5	2.2
20:4(n-6)	4	4.6	3.4	3.3	1.3	4	3.3	-	0.4
20:5(n-3)	13.9	6.1	8	7.4	10	8.2	11.4	10.1	3.9
22:4(n-3)	0.3	0.3	0.3	0.4	0.3	0.3	0.4	0.3	-
22:4(n-6)	0.9	1.3	0.9	0.9	0.3	1	1	0.3	0.4
22:5(n-3)	2.3	2.4	1.9	2.3	3.1	2.5	2.8	2.9	2.7
22:6(n-3)	13.2	17.7	17.7	16.5	16.8	13.6	18.8	16.5	11.3
Sum PUFA	**38.7**	**35.5**	**36.8**	**33.3**	**37.6**	**33.1**	**41.2**	**35.6**	**24.3**
Total	**100**	**100**	**100**	**96.5**	**100**	**100**	**100**	**97.2**	**99.1**
Cholesterol (mg/g)	**0.3**	**0.1**	**0.3**	**ND**	**0**	**0.1**	**0.1**	**ND**	**ND**

tr, trace (<0.1%)

Note: Values are for a single analysis and have been previously found to be reproducible to +/- 5%

Table 3. Proportion of major PUFA, sum of major PUFA (% of total fatty acids) and component ratios for shark liver oils and commercial ca

Fatty Acid	*Percentage Composition*			
	Gummy shark	**School shark**	**White-spotted Spurdog**	**MAXEPA**
20:4(n-6) AA	4	2.9	0.4	-
20:5(n-3) EPA	9.3	9.9	3.9	17.9
22:5(n-3) DPA	2.2	2.8	2.7	2.2
22:6(n-3) DHA	16.2	16.4	11.3	10.9
Sum of PUFA	37	37.3	24.3	41.2
ratio of EPA:DHA	0.57	0.60	0.35	1.64
ratio of omega 3: omega 6	4.19	8.85	7.88	7.45

AA, arachidonic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid

Marine Biology (1995) 122: 121–128

P. Virtue · R. E. Johannes · P. D. Nichols · J. W. Young

Biochemical composition of *Nyctiphanes australis* and its possible use as an aquaculture feed source: lipids, pigments and fluoride content

Received: 29 April 1994 / Accepted: 10 November 1994

Abstract *Nyctiphanes australis* contained, on a dry weight basis, an average of 52% crude protein and 5.0 to 9.5% lipid. The fatty acid profile of *N. australis* was markedly unsaturated, with a mean total ω3 fatty acid content of 48.6±2.4% of total fatty acids. *N. australis* contained high levels of the essential long-chain polyunsaturated fatty acids eicosapentaenoic (EPA, 20:5ω3) and docosahexaenoic (DHA, 22:6ω3), ranging from 16.6 to 36.5% and 11.1 to 24.8%, respectively. The concentration of total carotenoids ranged from 137 to 302 μg g^{-1} dry wt, with no significant differences in concentrations found with season or life stage. The carotenoids were comprised of 79.5% astaxanthin and 20.5% canthaxanthin. The lipid and pigment compositions of *N. australis* suggest that the species could serve as a suitable feed source for cultured salmonids. Like other euphausiids, *N. australis* contained high levels of fluoride, with a seasonal range between 277 and 3507 μg g^{-1} dry wt. The high fluoride levels found in *N. australis* would not detract from its potential as a feed source for salmonids because ingested fluoride is largely absorbed by the skeleton.

Introduction

Nyctiphanes australis is the principal euphausiid in continental shelf waters off south eastern Australia (Ritz and Hosie 1982), and is the most important single food item for a variety of abundant fishes in Tasmanian waters (O'Brien 1988). It was found, for example, to constitute 99.9% of the stomach contents of jack mackerel, *Trachurus declivis*, seined during a 19 mo study (Webb 1976). These fish, in turn, support a commercial fishery of several tens of thousands of tonnes per year (Williams et al. 1987). Simple calculations suggest that this predator alone must therefore consume several tens of thousands of tonnes of krill per year.

Nyctiphanes australis is also the principal food of a number of seabird species, including the short-tailed shearwater *Puffinus tenuirostris*, whose population numbers approximately 19 million adults plus immature individuals (Skira 1986). Taken together, these observations suggest that the annual production of this euphausiid may exceed 100 000 tonnes in these waters.

Krill have been shown to be of high nutritional value as components of aquaculture feed (Storebakken 1988, Shimizu et al. 1990), and are used extensively in feed for farmed salmonids and other fishes in Japan (Odate 1979) and Canada (Sloan and Fulton 1987). An important biochemical characteristic in this connection is the typical high concentration of the polyunsaturated fatty acids (PUFAs) in krill. PUFAs are essential nutritional requirements of various commercially raised fish and shellfish, including salmonids (Lall 1991); however, quantitative levels required by fish are not known. The fatty acid composition of fish is largely a reflection of that of their diet (Cowey and Sargent 1972). High PUFA content in fish is commercially desirable, as these fatty acids, in particular eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3), have been implicated in the prevention of human circulatory disorders (Bremner et al. 1989)

The carotenoid pigment astaxanthin is also typically present in high concentrations in krill (Funk and Hobson 1991). Astaxanthin and canthaxanthin are responsible for imparting the red colour to the flesh of salmonids and many other marine fish and shellfish species. Fish and other animals are unable to perform de novo synthesis of carotenoids, hence commercially farmed marine animals must obtain astaxanthin from their diets.

Tasmania supports a substantial salmonid farming industry which constitutes 2% of the world production (A.

Communicated by G. F. Humphrey, Sydney

P. Virtue (✉) · P. D. Nichols
CSIRO Divisions of Oceanography,
Marine Laboratories, GPO Box 1538, Hobart, Tasmania 7001, Australia

R. E. Johannes · J. W. Young
Division of Fisheries, Marine Laboratories, GPO Box 1538, Hobart, Tasmania 7001, Australia

Smithies, Salmonid Growers Association, personal communication). Due to the high local abundance of *Nyctiphanes australis*, the potential of this species as a constituent of fish feed in terms of lipid and astaxanthin content was investigated. Other comparable lipid studies of *N. australis* have been restricted to their analysis as stomach contents of predator species (Cheah and Hansen 1970; Bishop et al. 1976).

Fluoride analysis of *Nyctiphanes australis* was also undertake, since other krill are known to have unusually high concentrations of fluoride in their exoskeletons (Nicol and Stolp 1989) which makes them unacceptable for direct human consumption. This has raised the issue of their appropriateness as food for animals reared for human consumption (Grave 1981).

Materials and methods

Sample collection

Between November 1989 and January 1991, *Nyctiphanes australis* was sampled monthly with a 500 µm-mesh plankton net at various locations along the 80 m contour in Riedle Bay, east of Maria Island off the Tasmanian east coast. Additional sampling details are provided in Young et al. (1993). Individual krill for chemical analyses were picked from the krill haul immediately after emptying the net. About 2 to 10 mg (dry weight) of krill were used for each analysis (water content of krill ranged between 75 and 83%, n=12; and a mean of 79% was used to convert wet weights to dry weights). Within minutes of capture, krill samples were placed in vials and stored in liquid nitrogen. On return to the laboratory the samples were transferred to a storage dewar containing liquid nitrogen, and remained there until analysis.

Individual net hauls were invariably dominated by *Nyctiphanes australis* within a narrow size range. However, mean krill size varied considerably between net hauls, and has been classified as adults >10 mm, subadults 5 to 10 mm; and juveniles < 5 mm. These size classes correspond approximately to adult, post-larval and adolescent, calyptopis and furcilia stages (Sheard 1953; Young et al. 1993). Measurements were based on Mauchline's (1980) Standard 1.

Lipid extraction and analysis

Nyctiphanes australis were ground in chloroform and extracted quantitatively by the one-phase chloroform/methanol/water method of Bligh and Dyer (1959), as modified by White et al. (1979). After phase-separation, the lipids were recovered in the lower chloroform layer (solvents were removed in vacuo) and stored at -20°C in 1.5 ml vials fitted with teflon-lined caps. Fatty acid methyl esters (FAME) and free alcohols and sterols were formed by direct transesterification of an aliquot of the total lipids with methanol/chloroform/hydrochloric acid (10:1:1; 100 °C; 60 min). After cooling, 1 ml milli-Q-water was added and the products were extracted into hexane/chloroform (4:1). Solvents were removed under a stream of nitrogen and the alcohols and sterols were converted to their corresponding O-trimethylsilylethers by treatment with N.Obis(trimethylsilyl)trifluoroacetamide.

A portion of the total lipid extract was analysed for lipid composition with an Iatroscan MK III TH10 thin-layer chromatography/flame ionization detection (TLC/FID) analyser (Iatron Laboratories, Japan) (Volkman et al. 1986; Volkman and Nichols 1991). Gas chromatographic analysis of fatty acid methyl esters and sterols were performed on a Hewlett Packard 5890 GC equipped with a methyl silicone fused-silica capillary column and a flame-ionization detector. Operating instruction have been described in detail by Nichols et al. (1988). Fatty acids and sterols were identified by comparing mass spectral and retention-time data with that obtained for authentic and laboratory standards (see Nichols et al. 1989). Gas chromatography–mass spectroscopy (GC–MS) analysis of selected samples was performed on an HP 5890 GC and 5970 Mass Selective Detector fitted with a direct capillary inlet and a split/splitless injector.

Protein analysis

A sample of mixed krill (0.61 g wet wt) using individuals of different stages collected at various times of the year was analysed for crude protein. Total nitrogen was assayed by the Kjeldahl method (Association of Analytical Chemists 1984). Wet weight was converted to dry weight using a mean water content of 79.33%.

Pigment analysis

Total carotenoid content was estimated spectrophotometrically; a portion of the total lipid extract in 90% acetone was analysed for pigment composition using a Shimadzu UV-240 spectrophotometer (300 to 700 nm) (Ookubo and Matsuno 1985).

A representative sample of mixed krill using individuals of different stages collected at various times of the year was analysed using high-performance liquid chromatography, HPLC (Wright et al. 1991). Two peaks were resolved and identified by co-chromatography. One peak matched both the maximum wavelength (473 nm) and retention time (12 min) of the astaxanthin standard. The other peak co-chromatographed with known canthaxanthin from the blue green algae *Anabaena flos-aquae* with a maximum wave length of 467 nm and a retention time of 8 min (Wright et al. 1991). High-resolution MS data of the astaxanthin standard was acquired using a Kratos Concept ISQ with a direct probe inlet. Accurate mass data was acquired at 10000 resolution at a scan speed of 1 s per decade using perfluorokerosene as an internal reference. The standard was determined to contain pure astaxanthin.

Fluoride analysis

Fluoride was liberated from freeze-dried krill samples in a petri dish by acid digestion using perchloric acid saturated with Ag_2SO_4 (Lewis et al. 1987). The fluoride from this digest diffused into the petri dish lid which was coated with ethanolic sodium hydroxide solution (4% wt/vol. NaOH in 90% EtOH) while being heated at 50°C for 16 h. The alkaline mixture was dissolved in a 1:1 solution of water and TISAB II buffer consisting of NaCl and CDTA (cyclohexylenedinitrilo tetraacetic acid) in glacial acetic acid and water. The pH was adjusted to between 5.0 and 5.5 with 20% NaOH (Lewis et al. 1987). Fluoride concentration was measured with an Orion Model 96-09 combination fluoride electrode and a Radiometer Ion 85 pH/mV meter (resolution 0.1 mM).

Statistical analyses were performed by analysis of variance (ANOVA) using the "statistical analysis system" (Version 6.03, SAS Institute Inc 1988). Arcsine-transformation was done on all percentage data used for statistical analysis. Data are expressed as means ± standard deviation.

Results

The average crude protein content of *Nyctiphanes australis* was 52% of the total dry weight. The lipid content of *N. australis* ranged from 5.1 to 9.6% of the total dry weight, and no statistical difference was found between season or life stage. The lipid composition was dominated by polar lipids (including phospholipids and glycolipids) ranging from 68 to 86%. Triacylglycerols and sterols ranged between 5 to 21 and 5 to 8%, respectively (Table 1). No sig-

Table 1 *Nyctiphanes australis*. Dry weight, lipid class, total lipid, lipid-class ratios, cholesterol, carotenoid and fluoride levels as a function of season and life stage (*A* adult; *SA* subadult; *J* juvenile)

	1989				1990											Mean (SD)
	Nov	Dec			Feb	Mar					Apr	May	June	Sep	Nov	
	A	J	A	A	J	J	SA	A	A	A	SA	A	A	J	A	
Krill sample (g dry wt)	0.05	0.11	0.02	0.02	0.05	0.02	0.03	0.02	0.02	0.01	0.01	0.02	0.01	0.01	0.04	0.04 (0.03)
Lipid class (mg g^{-1} dry wt)																
triacylglycerol	12.2	5.3	9.8	4.8	6.2	9.9	8.8	14.9	11.4	14.0	10.9	16.1	4.1	14.0	14.6	11.2 (4.1)
free fatty acid	0.0	2.2	2.7	2.4	4.3	2.8	2.6	3.7	2.1	0.0	2.7	4.0	3.4	2.3	3.1	2.8 (0.8)
polar lipid	39.5	39.5	66.2	58.1	52.6	52.1	48.4	69.9	58.0	70.1	55.8	61.1	60.8	63.2	48.0	64.1 (10.0)
sterol	4.5	4.0	4.3	3.9	5.0	4.7	4.4	6.1	5.2	5.8	5.4	5.6	4.1	5.9	4.7	5.5 (0.7)
Total lipid (mg g^{-1} dry wt)	56.2	50.9	83.0	69.3	68.1	69.6	64.1	94.6	76.7	89.9	74.8	86.8	82.4	85.4	70.4	84.7 (11.4)
Cholesterol (mg g^{-1} dry wt)	1.1	0.4	3.9	2.0	1.1	0.3	1.9	2.4	2.4	2.4	2.4	2.4	0.8	2.6	1.9	1.9 (1.0)
Percentage lipid composition																
triacylglycerol	15.7	10.3	11.8	7.0	9.1	14.3	13.7	15.8	14.9	15.6	14.5	18.5	5.0	16.4	20.7	14.7 (4.3)
free fatty acid	0.0	4.3	3.3	3.5	6.4	4.1	4.0	3.9	2.7	0.0	3.6	4.6	4.1	2.7	4.4	3.8 (1.1)
polar lipid	78.5	77.6	79.7	83.9	77.3	74.8	75.4	73.9	75.7	77.9	74.5	70.4	86.0	74.0	68.1	87.1 (4.5)
sterol	5.8	7.8	5.2	5.6	7.3	6.8	6.9	6.4	6.8	6.5	7.3	6.5	5.0	6.8	6.7	7.5 (0.8)
Total lipid (% dry wt)	7.8	5.1	8.3	6.9	6.8	7.0	6.4	9.5	7.7	9.0	7.5	8.7	8.2	8.5	7.0	8.5 (1.1)
Triacylglycerol:polar lipid	0.2	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.1	0.2	0.3	0.2 (0.1)
Free fatty acid:polar lipid	0.0	0.1	0.0	0.0	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.5 (0.1)
Total carotenoid[a]																
μg g^{-1} dry wt	161	151	196	169	178	174	137	303	186	140	263	252	206	200	146	215 (46)
μg g^{-1} lipid dry wt	2080	2959	2361	2432	2616	2500	2130	3202	2420	1555	3517	2904	2494	2342	2068	2895 (489)
Fluoride (μg g^{-1} dry wt)	531	548	1079	1084	684	3506.5	277	1198	665	2521.3	3212	1077	3500	1930	883	1556 (1129)

[a] Carotenoid included both astaxanthin and canthaxanthin at ≃ 79.5 and 20.5%, respectively

nificant seasonal differences were detected in the lipid class composition of the samples.

Cholesterol was the major sterol with an average concentration over season and life stage of 1.9±1.0 mg g^{-1} dry wt. Only traces of desmosterol (cholesta-5, 24-dien-3β-ol) and brassicasterol (24-methylcholesta-5,22E-dien-3β-ol) were detected. Non-esterified (free) fatty acids constituted only minor components of the total lipid (3.8%±1.1, Table 1). This relatively low percentage of free fatty acid indicates adequate storage of the samples prior to extraction (Saether et al. 1986).

The saturated fatty acid 16:0 and the long-chain polyunsaturated fatty acids eicosapentaenoic (EPA, 20:5ω3) and docosahexaenoic (DHA, 22:6ω3) dominated the fatty acid composition of *Nyctiphanes australis* (Table 2). The two major polyenoic acids, EPA and DHA, ranged from 16.6 to 36.5 and 11.1 to 24.8% of the total fatty acids, respectively. The fatty acid profile was markedly unsaturated, with the mean total ω3 fatty acid being 48.6±2.4%. Significant differences were found in the levels of EPA and DHA between life stages. Both adult and subadult life stages differed significantly from juvenile krill in levels of these two PUFAs. Juveniles were found to have a higher mean percent EPA (23.6%) than the adults (17.7%) and subadults (19.5%). Juveniles had lower mean percent DHA (18.1%) compared with adults (22.3%) and subadults (23.7%). These differences were probably due to the influence of one sample (September 1990) which was significantly different from the total data set. No significant differences were found however in the total ω3 fatty acids between season and life stages.

Both astaxanthin and canthaxanthin were detected in *Nyctiphanes australis*, with total carotenoid levels ranging from 137 to 303 μg g^{-1} dry wt. Levels of astaxanthin and canthaxanthin were 79.5 and 20.5%, respectively. Fluoride concentrations ranged between 277 and 3507μg g^{-1} dry wt. No significant differences in either carotenoid or fluoride levels were evident between season or life stage (Table 1).

Discussion

Marine zooplankton tend to have lower lipid concentrations at lower latitudes (Sheard 1953), and *Nyctiphanes australis* is no exception. The total lipid in *N. australis* (8.5% dry wt) was less than values which characterize high-latitude krill species. *Meganyctiphanes norvegica*, *Thysanoessa raschii* and *T. inermis*, for example, all of which are found in northern polar regions, attain seasonal peak lipid contents of about 40, 40 and 50% dry wt, respectively (Falk-Petersen et al. 1981), and concentrations vary seasonally by roughly threefold, fourfold, and fourfold respectively (Saether et al. 1986). Lipid levels of the Antarctic euphausiid *Euphausia crystallorophias* range between 10% dry weight in November and 35% dry weight in May (Littlepage 1964). All these polar species depend on lipid stores to survive periods of low plankton production in the winter. Lipid levels of the Antarctic krill *E. superba* collected between spring and autumn are also highly variable, and range from 3 to 36% dry weight (Clarke 1980; Ellingsen and Mohr 1981; Saether et al. 1986; Hagen 1988; Virtue et al. 1993). It has been proposed that this latter species may not rely solely on lipid sources for overwintering (Ikeda and Dixon 1982). Unlike high-latitude species, little seasonal variation in lipid content was detected in *N. australis* in the present study.

All samples of *Nyctiphanes australis* analysed in this study had relatively low levels of the storage lipid, triacylglycerols (11%), compared to other euphausiids such as *Meganyctiphanes norvegica* (53%), *Thysanoessa raschii* (44%) and *Euphausia superba* (37%) (Sargent and Falk-Petersen 1981; Virtue et al. 1993). The majority of lipid in *N. australis* was in the form of structural components. Phospholipids and cholesterol are structural elements of the plasma membrane, whereas storage lipids such as triacylglycerols and wax esters are used as energy stores (Lee et al. 1970; Sargent 1976).

The similarity in lipid content and composition of these samples would imply a relatively constant available food supply throughout our sampling period. Harris et al. (1987), however, reported season fluctuations in phytoplankton in this region, with bloom conditions during spring. *Nyctiphanes australis* is omnivorous (Mauchline 1980), and Ritz et al. (1990) concluded from a study of stomach contents analysed quantitatively throughout the year, that *N. australis* was an opportunistic omnivore/detritivore. Young et al. (1993) reported no significant relationship between length and weight of *N. australis* with season, using samples collected from the same stations as those of the present study. Due to their ability to exploit a variety of food sources, *N. australis* is able to maintain physiological status in terms of lipid content and composition under conditions of reduced phytoplankton.

Although physiological condition appears to be maintained in *Nyctiphanes australis* throughout the year, a response to environmental conditions may be evident in a more dynamic sense. Young et al. (1993) reported significant variation both seasonally and interannually in density, biomass and population structure of *N. australis*. This variation was attributed to regional oceanographic conditions driving phytoplankton production. Biomass estimates may also be influenced by both seasonal predation upon *N. australis* and the fact that this species may live at depths out of plankton-tow range during the winter months (Young et al. 1993).

In terms of mariculture feed, the total lipid level of *Nyctiphanes australis* is near the optimum range used for salmonids. Fish and crustaceans fed diets high in total lipid accumulated excess lipid which was deposited as visceral fat. This excess is discarded as waste during processing. The weight of the mesenteric adipose tissue in salmonid fish has been found to increase with increasing dietary lipid (Lin et al. 1977; Takeuchi et al. 1978).

A number of samples analysed which were not included in this data set contained wax esters that ranged from 2 to 7% of the total lipid. All these samples consisted of very

Table 2 *Nyctiphanes australis*. Percentage fatty acid composition as a function of season and life stage (*A* adult; *SA* subadult; *J* juvenile; *other* includes all categories (<0.5%)

Fatty acid	1989				1990											Mean (SD)
	Nov	Dec			Feb	Mar					Apr	May	June	Sep	Nov	
	A	J	A	A	J	J	SA	A	A	A	SA	A	A	J	A	
14:0	5.5	4.9	5.3	4.2	4.4	6.1	5.6	5.9	5.4	5.8	5.8	6.2	3.5	3.5	6.0	5.2 (0.9)
15:1ω8	0.1	0.1	0.2	0.0	0.2	0.2	0.3	0.0	0.3	0.4	0.0	0.0	0.7	0.0	0.0	0.2 (0.2)
15:0	1.1	1.2	0.9	0.5	1.3	1.2	1.0	1.0	1.0	1.7	1.0	1.5	1.2	0.3	0.9	1.1 (0.3)
16:4ω1	0.6	0.4	0.6	0.5	0.4	0.5	0.3	0.4	0.3	0.5	0.5	0.8	0.5	1.3	0.4	0.5 (0.2)
16:3ω4	0.2	0.2	0.1	0.1	0.2	0.2	0.1	0.1	0.1	0.4	0.0	0.1	0.2	0.6	0.1	0.2(0.1)
16:1ω7	2.6	4.3	2.7	2.6	2.6	2.9	1.6	1.7	1.7	2.3	2.2	2.2	2.1	7.0	2.5	2.7 (1.4)
16:1ω5	0.7	0.4	0.7	0.3	0.4	0.4	0.3	0.3	0.2	0.6	0.3	0.1	0.4	0.5	1.0	0.4 (0.2)
16:0	25.2	24.8	22.5	21.3	23.0	23.1	21.6	21.2	20.9	23.4	21.4	20.9	23.5	20.2	21.4	22.3 (1.5)
17:0	0.9	0.9	0.8	0.5	1.0	1.0	0.9	0.9	0.9	1.2	0.8	1.1	1.1	0.4	0.9	0.9 (0.2)
18:4ω3	5.1	2.7	5.5	3.1	2.9	4.3	2.9	4.7	3.5	2.6	4.2	5.3	4.0	1.8	7.3	4.0 (1.4)
18:2ω6	3.6	3.0	3.5	2.5	3.2	3.5	3.5	4.1	3.5	3.5	4.0	3.2	3.0	1.2	2.8	3.2 (0.7)
18:3ω3	3.8	2.3	3.2	2.0	3.4	3.1	2.5	3.7	2.6	3.2	3.5	3.4	3.2	0.5	3.0	2.9 (0.8)
18:1ω9	6.5	6.2	6.8	6.8	7.9	7.0	8.6	8.2	8.2	7.9	8.5	7.3	7.8	5.3	5.6	7.2 (1.0)
18:1ω7	3.2	3.9	3.3	3.8	3.2	3.3	2.6	2.8	2.7	3.8	3.1	3.6	3.3	5.8	2.9	3.4 (0.8)
18:0	2.3	2.3	2.2	1.6	2.9	2.7	2.2	2.2	2.3	2.3	2.1	2.1	2.7	2.2	1.6	2.2 (0.4)
20:4ω6	0.0	0.0	0.0	2.5	0.0	0.0	0.0	1.8	2.3	1.9	1.8	0.0	1.9	0.0	0.0	0.8 (0.3)
20:5ω3	17.1	20.9	17.7	21.3	19.3	18.8	20.6	16.6	18.4	16.6	17.1	17.5	16.7	36.5	17.9	19.5 (4.9)
20:4ω3	0.5	0.4	0.5	0.3	0.5	0.3	0.2	0.4	0.2	0.4	0.3	0.3	0.4	0.5	0.6	0.4 (0.1)
20:2ω6	0.3	0.2	0.2	0.1	0.3	0.2	0.2	0.6	0.1	0.3	0.0	0.2	0.0	0.0	0.2	0.2 (0.1)
20:1ω9	0.5	0.7	0.4	0.5	0.6	0.4	0.3	0.2	0.1	0.4	0.3	0.1	0.7	0.5	0.9	0.5 (0.2)
22:6ω3	18.9	19.2	21.8	24.8	21.3	20.6	24.4	22.4	24.4	20.5	22.9	23.0	22.0	11.1	22.6	21.3 (3.3)
22:5ω3	0.4	0.4	0.5	0.3	0.4	0.3	0.4	0.5	0.3	0.4	0.2	0.5	0.6	0.8	0.5	0.4 (0.1)
other	0.5	0.4	0.3	0.0	0.5	0.0	0.0	0.2	0.0	0.0	0.0	0.3	0.2	0.0	0.4	0.2 (0.1)
16:1ω7/16:0	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.3	0.1	0.1 (0.1)
18:1ω7/18:1ω9	0.5	0.6	0.5	0.6	0.4	0.5	0.3	0.3	0.3	0.5	0.4	0.5	0.4	1.1	0.5	0.5 (0.2)
total ω7	5.8	8.3	5.9	6.4	5.8	6.1	4.2	4.5	4.3	6.1	5.2	5.7	5.4	12.8	5.4	6.1 (2.1)
total ω3	45.8	45.8	49.3	51.8	47.9	47.4	51.0	48.2	49.5	43.7	48.2	50.0	46.9	51.2	52.0	48.6 (2.4)

small individuals, and microscopic examination revealed that they also contained significant numbers of copepods, unlike samples consisting of large euphausiids which could be readily sorted. Analyses of samples containing copepods are not presented here. These observations however, may explain previous conflicting reports concerning the presence or absence of wax esters in *Nyctiphanes australis*: Bishop et al. (1983) reported significant concentrations in this species, whereas Cheah and Hansen (1970) found none.

Total ω3 fatty acids constituted almost half the total fatty acids found in *Nyctiphanes australis*, with the two essential polyenoic acids, EPA and DHA dominating. These levels compared well with those found in partially digested krill from stomachs of slender tuna (Bishop et al. 1976). Levels of both EPA and DHA were 25 to 30% higher than those reported for polar species such as *Meganyctiphanes norvegica, Thysanoessa raschii, T. inermis* (Sargent and Falk-Petersen 1981), and *Euphausia superba* (Virtue et al. 1993). The food source of *N. australis* may contain substantial quantities of both eicosapentaenoic and docosahexaenoic acids. Alternatively/in addition, *N. australis* may possess some ability to elongate and desaturate dietary fatty acids.

The superiority of long-chain over short-chain C_{18} PUFAs in maintaining growth has been reported in nutritional studies for marine fish (reviewed by Sargent and Whittle 1981). It has been suggested that carnivorous marine fish, which have natural diets high in EPA and DHA, may not have evolved or may perhaps have lost the enzymatic pathways for the elongation and desaturation of shorter-chain PUFAs to these longer ones (Cowey and Sargent 1977; Sargent and Whittle 1981). ω3 fatty acids are essential for salmonid development (Ashton et al. 1993). Species such as rainbow trout (*Oncorhynchus mykiss*) require ω-3 rather than ω-6 fatty acids (Castell et al. 1972). Dietary deficiencies in ω-3 fatty acids in salmonid fish cause physiological dysfunction of developing fish and early embryonic mortality (Watanabe 1982; Leray et al. 1985). Because *Nyctiphanes australis* contains very high levels of long-chain ω3 PUFAs, it would therefore be an attractive food source for mariculture species.

The crude protein content of *Nyctiphanes australis* (52%) was typical of that found in euphausiids (reviewed by Mauchline 1980). Crude protein is not a measure of metabolizable energy. Krill carapace, which is a nitrogenous polysaccharide, represents a portion of crude protein. Amino acid protein constitutes 78% of the crude protein in whole *Euphausia superba* (Pierce et al. 1969; Siebert et al. 1980). Amino acid content was not analysed in *N. australis* in the present study; however, assuming levels similar to those found in *E. superba*, protein in terms of metabolizable energy would be ~40%. A survey of the quality of various fish meals available to the Tasmanian salmonid industry reported percentage protein in the range of 60 to 70% (Foster 1991). The protein content of *N. australis* would be considered adequate in terms of a mariculture feed, although supplemental protein might be required.

Although, as in other euphausiids, the fluoride concentration in whole *Nyctiphanes australis* would be considered too high for both human consumption and stock feed, this species would be suitable as mariculture feed. The United States Food and Drug Administration (USFDA) allowance for human consumption is 100 $\mu g\ g^{-1}$ of fluoride per day (Budzinski et al. 1985). In both vertebrates and invertebrates, fluoride is accumulated in the skeletal structures. Grave (1981) reported that the elevated fluoride concentration in salmonids fed a pure krill diet was restricted largely to skeletal tissue. Fluoride content of muscle tissue increased slightly, but did not exceed concentrations reported in various wild salmonid species and was < 2% of the USFDA limit for human consumption. Oehlenschlager and Manthey (1982) investigated the fluoride content of Antarctic fish, and found no differences in fish feeding on krill and fish feeding predominantly on other fish. They found fish muscle to contain 2 $\mu g\ g^{-1}$ fluoride, which is similar to that of fishes in other waters; however, the fluoride level found in the bone tissue of these fish was in the order of 600 to 1200 $\mu g\ g^{-1}$. The flesh of mariculture species such as salmonid fed on a krill-based diet would not, therefore, be expected to contain high levels of fluoride. Rather, if accumulated, fluoride might be expected to be found in the skeletal material.

A large variation in fluoride levels in *Nyctiphanes australis* was found in this study. Adelung et al. (1987) reported >99% of fluoride to be concentrated in the cuticle of euphausiids (2600 $\mu g\ g^{-1}$ dry wt in *Euphausia superba* and 3300 $\mu g\ g^{-1}$ in *Meganyctiphanes norvegica)*. As fluoride accumulates mainly in the chitinous exoskeleton of crustaceans and is found in very low concentrations in the muscle and soft tissue (50 to 100 $\mu g\ g^{-1}$; Szewielow 1981), a substantial fluctuation within the moult cycle would be expected in *N. australis*.

Astaxanthin is the major carotenoid in many crustaceans and was found to be the dominant carotenoid in *Nyctiphanes australis* in this study. *N. australis* is somewhat richer in this pigment than *Euphausia superba*, which was found to have a mean astaxanthin content of 94 $\mu g\ g^{-1}$ dry wt (converted from wet weight using 77% tissue water content for this species; Clarke 1980). Carotenoids such as astaxanthin and canthaxanthin are used as pigmenting agents by the mariculture industry. Carotenoids cannot be synthesised de novo by salmonids, and hence dietary supplements are required in net-pen reared fish (Storebakken and No 1992).

Euphausia superba, which is rich in carotenoids and contains mainly (3R,3'R)-astaxanthin diester, was used successfully to enhance integument pigmentation of cultured yellow tail and sea bream (Fujita et al. 1983; Maoka et al. 1985; Miki et al. 1985). Shigeru et al. (1987) fed oil extracted from *Euphausia superba* to 180 g coho salmon (*Oncorhynchus kisutch*) for 8 wk, and reported marked flesh pigmentation which was found to consist mainly of astaxanthin.

Physiological conditions vary within and between species, and the need for size- and sex-specific research is necessary to determine the rate of deposition of carotenoids in

fish flesh. Based on our results, *Nyctiphanes australis* is a potential astaxanthin source for use in salmonid culture. Feeding studies are warranted to confirm the commercial use of this species as a pigmentation agent. Other factors relevant to the species' potential commercial value will be discussed elsewhere (Johannes in preparation).

Acknowledgements This research was supported in part by Fisheries Research and Development Corporation (FRDC). We gratefully acknowledge M. Sands of the Institute of Antarctic and Southern Ocean Studies, University of Tasmania, for assistance with fluoride analysis. Dr S. Wright of the Antarctic Division is thanked for providing expertise with HPLC pigment analysis. C. Bobbi and B. Barker of CSIRO Division of Fisheries helped with the fieldwork. M. Brown, G. Dunstan of CSIRO and two anonymous reviewers provided helpful criticisms of the manuscript.

References

Adelung D, Buchholz F, Culik B, Keck A (1987) Fluoride in tissues of krill *Euphausia superba* Dana and *Meganyctiphanes norvegica* M. Sars in relation to the moult cycle. Polar Biol 7:43–50

Ashton HJ, Farkvam DO, March BE (1993) Fatty acid composition of lipids in the eggs and alevins from wild and cultured chinook salmon (*Orcorhynchus tshawytscha*) Can J Fish aquat Sciences 50:648–655

Association of Official Analytical Chemists (AOAC) (1984) Official methods of analytical chemists. 14th edn. Horwitz W (ed) AOAC, Washington, DC

Bishop DG, James DG, Olley J (1976) Lipid composition of slender tuna (*Allothunnus fallai*) as related to lipid composition of their feed (*Nyctiphanes australis*) J Fish Res Bd Can 33:1156–1161

Bishop DG, Ritz DA, Hosie GW, Kenrick J R, Olley J (1983) Fatty acid composition of the lipids of *Puffinus tenuirostris* (Temminck) in relation to its diet. J exp mar Biol Ecol, 71:17–26

Bligh E, GDyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–917

Bremner HA, Volkman JK, Krasnicki T, Gibson R (1989) The good oil: nutritionally important oils in Tasmanian fish. Aust Fish 48 (5):28–29

Budzinski E, Bykowski P, Dutkiewicz D (1985) Possibilities of processing and marketing of products made from Antarctic krill. FAO Fish tech Pap 268:1–46

Castell JD, Lee DJ, Sinnhuber RO (1972) Essential fatty acids in the diet of rainbow trout (*Salmo gairdneri*): lipid metabolism and fatty acid composition. J Nutr 102:93–100

Cheah CC, Hansen IA (1970) Wax esters in the stomach oil of petrels. Int J Biochem 1:198–202

Clarke A (1980) The biochemical composition of krill, *Euphausia superba* Dana, from South Georgia. J exp mar Biol Ecol 43:221–236

Cowey CB, Sargent JR (1972) Fish nutrition. Adv mar Biol 10:383–492

Cowey CB, Sargent JR (1977) Minireview. Lipid nutrition in fish. Comp Biochem Physiol 57B:269–273

Ellingsen TE, Mohr V (1981) Lipids in Antarctic krill. Composition and *post mortem* changes. In: Marcuse R (ed) Proceedings of the Eleventh Scandinavian Symposium on Lipids. SIK, Gothenburg, Sweden, pp 110–116

Falk-Petersen SR, Gatten R, Sargent JR, Hopkins CCE (1981) Ecological investigations on the zooplankton community in Balsfjorden, northern Norway: seasonal changes in the lipid class composition of *Meganyctiphanes norvegica* (M. Sars), *Thysanoessa raschii* (M. Sars) and *T. interims* (Krøyer) J exp mar Biol Ecol 54:209–224

Foster CK (1991) A survey of the quality of fishmeal available to the Tasmanian salmonid industry. In: Valentine P (ed) Proceedings of the SALTAS Research Review Seminar. CSIRO Marine Laboratories, Hobart, Tasmania, pp 165–175

Funk VA, Hobson LA (1991) Temporal variations in the carotenoid composition and content of *Euphausia pacifica* Hansen in Saanich Inlet, British Columbia. J exp mar Biol Ecol 148:93–104

Fujita T, Satake M, Watanabe T, Kitajima C, Miki W, Yamaguchi K, Konosu S (1983) Pigmentation of cultures of yellowtail with krill oil. Bull Jap Soc scient Fish 66:255–264

Grave H (1981) Fluoride content of salmonids fed on Antarctic krill. Aquaculture, Amsterdam 24:191–196

Hagen W (1988) Zur Bedeutung der Lipide im antarktischen Zooplankton [On the significance of lipids in Antarctic zooplankton.] Ber Polarforsch 49:1–129

Harris GP, Nilsson C, Clementson L, Thomas D (1987) The water masses of the east coast of Tasmania: seasonal and interannual variability and the influence of phytoplankton biomass and productivity. Aust J mar Freshwat Res 38:569–590

Ikeda T, Dixon P (1982) Body shrinkage as a possible over-wintering mechanism of the Antarctic krill, *Euphausia superba* Dana. J exp mar Biol Ecol 62:143–151

Lall SP (1991) Salmonid nutrition and feed production. Tech Rep Fish aquat Sciences, Can 1831:107–123

Lee RF, Nevenzel JC, Paffenhöfer GA, Benson AA (1970) The metabolism of wax esters and other lipids by the marine copepod, *Calanus helgolandicus*. J Lipid Res 11:237–240

Leray C, Nanotte G, Rouband P, Leger C (1985) Incidence of *n*3 essential fatty acid deficiency on trout reproductive processes. Reprod Nutr Dév 25:567–581

Lewis TL, Stumpers AC, Davies DM (1987) Analysis of fluoride in foodstuffs and krill. In: Fayden GS, Jeffrey A (eds) Proceedings of the 9th Australian Symposium of Analytical Chemists. Queensland Department of Primary Industries, Brisbane, pp 168–171

Lin H, Romsos DR, Tack PI, Leveille GA (1977) Influence of dietary lipid on lipogenic enzyme activities in coho salmon (*Oncorhynchus kisutch* (Walbaum)) J Nutr 107:846–854

Littlepage J (1964) Seasonal variation in lipid content of two Antarctic marine crustaceans. In: Biologie antarctique. Carrick R, Holdgate MW, Prevost J (eds), First SCAR Symposium, Paris 2.–8. 9. 1962. Hermann, Paris, pp 463–470

Maoka T, Katsuyama M, Kaneko N, Matsuno T (1985) Stereochemical investigations of carotenoids in the Antarctic krill *Euphausia superba*. Bull Jap Soc scient Fish 51:1671–1673

Mauchline J (1980) The biology of euphausiids. Adv mar Biol 18:373–554

Miki W, Yamaguchi K, Konosu S, Takane T, Satake M, Fujita T, Kuwabara H, Shimeno S, Takeda M (1985) Origin of tunaxanthin in the integument of yellowtail (*Seriola quinqueradiata*) Comp Biochem Physiol 80B:195–201

Nichols PD, Palmisano AC, Rayner MS, Smith GA, White DC (1989) Changes in the lipid composition of Antarctic sea-ice diatom communities during a spring bloom: an indication of community physiological status. Antarctic Sci 1:133–140

Nichols PD, Volkman JK, Palmisano AC, Smith AS, White DC (1988) Occurrence of an isoprenoid C_{25} diunsaturated alkene and high neutral lipid content in Antarctic sea-ice diatom communities. J Phycol 24:90–96

Nicol S, Stolp M (1989) Sinking rates of cast exoskeletons of Antarctic krill (*Euphausia superba* Dana) and their role in the vertical flux of particulate matter and fluoride in the Southern Ocean. Deep-Sea Res 36:1753–1762

O'Brien DP (1988) Surface schooling behaviour of the coastal krill *Nyctiphanes australis* (Crustacea: Euphausiacea) off Tasmania, Australia. Mar Ecol Prog Ser 12:219

Odate K (1979) An euphausiid crustacea exploited along the Sanriku and Joban coast. Bull Tohoku reg Fish Res Lab 40 (Jan):15–25

Oehlenschlager J, Manthey M (1982) Fluoride content of antarctic marine animals caught off Elephant Island. Polar Biol 1:125–127

Ookubo M, Matsuno T (1985) Carotenoids of sea squirts. II. Comparative biochemical studies of carotenoids in sea squirts. Comp Biochem Physiol 81:137–141

Pierce RW, Van Veen J, Olcott HS (1969) Proximate and lipid analyses of krill (*Euphausia* species) and red crab (*Pleuroncodes planipes*) J agric Fd Chem 17:367–369

Ritz DA, Hosie GW (1982) Production of the euphausiid *Nyctiphanes australis* in Storm Bay, South-Eastern Tasmania. Mar Biol 68:103–108

Ritz DA, Hosie G, Kirkwood RJ (1990) Diet of *Nyctiphanes australis* Sar (Crustacea: Euphausiacea) Aust J mar Freshwat Res 41:365–374

Saether O, Ellingsen TE, Mohr V (1986) Lipids of North Atlantic krill. J Lipid Res 27:274–285

Sargent JR (1976) Structure, metabolism and function of lipids in marine organisms. In: Malins DC, Sargent JR (eds) Biochemical and biophysical perspectives in marine biology. Vol. 3. Academic Press, London, pp 149–212

Sargent JR, Falk-Petersen S (1981) Ecological investigations on the zooplankton community of Balsfjorden, northern Norway: lipids and fatty acids in *Meganyctiphanies norvegica*, *Thysanoessa raschii* and *T. inermis* during mid-winter. Mar Biol 62:131–137

Sargent JR, Whittle KJ (1981) Lipids and hydrocarbons in the marine food web. In: Longhurst AR (ed) Analysis of marine ecosystems. London, Academic Press, pp 491–533

SAS Institute Inc (1988) SAS/STAT™ statistical guide for personal computers. Users guide. Release 6.03 edn. SAS Institute Inc, Cary, North Carolina

Sheard K (1953) Taxonomy, distribution and development of Euphausiacea (Crustacea). Rep BANZ antarct Res Exped (Ser B) 8:1–72

Shigeru A, Tetsu M, Wataru M, Katsumi Y, Shoji K, Satake M, Takao F (1987) Pigmentation of juvenile coho salmon with carotenoid oil extracted from antarctic krill. Aquaculture, Amsterdam 66:255–264

Shimizu C, Ibrahim A, Tokoro T, Shirakawa Y (1990) Feeding stimulation in sea bream, *Pagrus major*, fed diets supplemented with Antarctic krill meals. Aquaculture, Amsterdam 89:43–53

Siebert G, Kuhl J, Hannover R (1980) Nutritional experiments with krill. In: Connell JJ (ed) Advances in fish science and technology. Fishing News Books, Farnham, pp 332–334

Skira IJ (1986) Food of the short-tailed shearwater, *Puffinus tenuirostris*, in Tasmania. Aust Wildl Res 13:481–488

Sloan NA, Fulton J (1987) 1. Plankton – euphausiids (trawl fishery). Tech Rep Fish aquat Sciences, Can 1576:9–15

Storebakken T (1988) Krill as a potential feed source for salmonids. Aquaculture, Amsterdam 70:193–205

Storebakken T, No HK (1992) Pigmentation of rainbow trout. Aquaculture, Amsterdam 100:209–229

Szewielow A (1981) Fluoride in Antarctic krill (*Euphausia superba* Dana). Meeresforsch Rep mar Res 28:244–246 (Ber dt wiss Kommn Meeresforsch)

Takeuchi T, Watanabe T, Ogino C. (1978) Supplementary effect of lipids in a high protein diet of rainbow trout. Bull Jap Soc scient Fish 44:677–681

Virtue P, Nichols P, Nicol S, McMinn A, Sikes E (1993) The lipid composition of *Euphausia superba* Dana in relation to the nutritional value of *Phaeocystis pouchetii* (Hariot) Lagerheim. Antarctic Sci 5:169–177

Volkman JK, Everitt DA, Allen DI (1986) Some analyses of lipid classes in marine organisms sediments and seawater using thin-layer chromatography–flame ionisation detection. J Chromat 6:147–162

Volkman JK, Nichols PD (1991) Applications of thin layer chromatography–flame ionization detection to the analysis of lipids and pollutants in marine and environmental samples. J planar Chromat 4:19–26

Watanabe T (1982) Lipid nutrition in fish. Comp Biochem Physiol 73B:3–15

Webb BF (1976) Aspects of the biology of jack mackerel, *Trachurus declivis* (Jenyns), from south east Australian waters. Tasm Fish Res 10:1–17

White DC, Davis WM, Nickels JS, King JD, Bobbie RJ (1979) Determination of the sedimentary microbial biomass by extractable lipid phosphate. Oecologia 40:51–62

Williams H, Pullen G, Kucerans G, Waterworth C (1987) The jack mackerel purse seine fishery in Tasmania, 1986–1987. Tech Rep Dep Sea Fish, Tasm 19:1–32

Wright SW, Jeffrey SW, Mantoura RFC, Llewellyn T, Bjornland T, Repeta D, Welschmeyer N (1991) Improved HPLC method for the analysis of chlorophylls and carotenoids from marine phytoplankton. Mar Ecol Prog Ser 77:183–196

Young JW, Jordan AR, Bobbi C, Johannes RE, Haskard K, Pullen G (1993) Seasonal and interannual variations in krill (*Nyctiphanes australis*) stocks and their relationship to the fishery for jack mackerel (*Trachurus declivis*) off eastern Tasmania, Australia. Mar Biol 116:9–18

Lipids and Buoyancy in Southern Ocean Pteropods

Charles F. Phleger[a,*], Peter D. Nichols[b], and Patti Virtue[b]

[a]Department of Biology, San Diego State University, San Diego, California, 92182, and [b]CSIRO Division of Marine Research and Antarctic Co-operative Research Centre, Hobart, Tasmania, 7001, Australia

ABSTRACT: The lipids of *Clione limacina*, a Southern Ocean pteropod (order Gymnosomata), contain 28% diacylglyceryl ether (DAGE) (as percentage of total lipid) whereas the pteropod *Limacina helicina* (order Thecosomata) lacks DAGE. The alkyl glyceryl ether diols (1-*O*-alkyl glycerols, GE) of *Clione* DAGE are dominated by 16:0 (60%) and 15:0 (21%), in contrast with deep-sea shark liver DAGE, which is dominated by 18:1 GE. The fatty acid profiles of *Clione* and *Limacina* are similar (28–32% polyunsaturated, 26–34% monounsaturated) as are the sterols, which include 24-methylenecholesterol, trans-dehydrocholesterol, cholesterol, and desmosterol. This finding probably reflects the fact that *Limacina* is the major food source for *Clione*. *Spongiobranchaea australis*, another Southern Ocean pteropod (order Gymnosomata), has 0.9–1.7% DAGE, but has less lipid (3.3–4.8 mg/g lipid, wet weight) than *Clione* (50.8 mg/g lipid, wet weight). We propose a buoyancy role for DAGE in *Clione* since *Limacina* has bubbles for flotation which *Clione* lack; DAGE provides 23% more uplift than triacylglycerol at a concentration of 1.025 g/mL seawater.
Lipids 32, 1093–1100 (1997).

Marine zooplankton maintain buoyancy by diverse strategies including hypotonicity, increased surface area, swimming, gas bubbles, and storage of low-density materials such as lipid (0.86–0.93 g/mL). The most important storage lipids in marine zooplankton are wax esters (WE) and triacylglycerol (TAG) (1). WE may be concentrated in polar and deep ocean copepods, where they are utilized primarily as long-term energy reserves (2). TAG, however, are major short-term energy reserve molecules in most zooplankton. WE have a lower metabolic turnover rate than TAG, and thus may have a more important function in buoyancy than TAG (3). The density of WE (0.86 g/mL at 21°C) is less than TAG (0.93 g/mL at 21°C). Diacylglyceryl ether (DAGE), although not a major lipid in zooplankton, also has a lower metabolic turnover rate than TAG and a lower density (0.89 g/mL at 21°C). DAGE also generates 23% more upthrust than TAG (0.127 g upthrust/g lipid vs. 0.103 g upthrust/g lipid in seawater of 1.025 g/mL density) (4).

Lipid composition is also used to understand and identify food web interactions (5,6). The Southern Ocean has a complex food web including phytoplanktivorous herbivores fed upon by birds, fish, squid, seals, and baleen whales (7). Sterols (ST) and fatty acids are excellent biomarkers for food chain studies (8). The herbivorous Thecosome pteropod *Limacina helicina* is very common in the Southern Ocean, as in other oceans (9). It is fed upon by the carnivorous Gymnosome pteropod, *Clione limacina*, another abundant holoplanktonic Opisthobranch mollusk. *Spongiobranchaea australis* is another highly predaceous Gymnosome pteropod, which also feeds exclusively on a Thecosome pteropod (*Clio pyramidata*).

Limited lipid compositional data are available for these Southern Ocean pteropods. The purpose of this study is to report the use of DAGE as a possible buoyancy lipid in the pteropod *C. limacina*, and to clarify and further understand food web interactions by examining pteropod ST and fatty acids as key biochemical factors.

MATERIALS AND METHODS

Sample description. The pteropods *C. limacina* and *L. helicina* were collected by Isaacs-Kidd midwater trawl from the R/V *Yuzhmorgeologia* on January 23 and 22, 1996, respectively. These samples were obtained as part of the Antarctic Marine Life Research Field Study conducted annually in the Elephant Island region of the Antarctic peninsula (10,11). *Clione limacina* was trawled from station A-04 (off Admiralty Bay, King George Island) at 62°14.4′ S and 57°58.5′ W. *Limacina helicina* was trawled from station AB-03 (within Admiralty Bay). In both cases the net was towed to 170 m depth for about 30 min. Pteropods were frozen at −10°C as soon as possible after sorting and counting on board ship. They were then transported frozen by air to the CSIRO Division of Marine Research where they were maintained at −60°C prior to analysis. *Spongiobranchaea australis* was collected by rectangular midwater trawl (to 195 m) from the R/V *Aurora Australis* on Expedition Broke on March 12, 1996, from the Adélie coast (within Wilkes land) region of East Antarctica (63°26.27′ S and 138°59.21′ W). They were

*To whom correspondence should be addressed.
E-mail: phleger@sunstroke.sdsu.edu.

Abbreviations: DAGE, diacylglyceryl ether; FFA, free fatty acids; FID, flame-ionization detector; GC, gas chromatography; GE, 1-*O*-alkyl glycerols; MS, mass spectrometric; PL, phospholipid; PUFA, polyunsaturated fatty acids; ST, sterols; TAG, triacylglycerols; TLC, thin-layer chromatography; TMS, trimethylsilyl; WE, wax esters.

frozen in liquid nitrogen immediately and transported frozen to the CSIRO Hobart laboratory for analysis. Individuals of *C. limacina* were pooled for extraction owing to the limited sample size (8–9 individuals weighed 0.25 g wet weight). It was possible to pool 20–25 individuals of *L. helicina* for three extractions owing to a larger sample size. Duplicate analyses of two individuals of the considerably larger *S. australis* were performed.

Lipid extraction. Samples were quantitatively extracted using a modified Bligh and Dyer (12) one-phase methanol/chloroform/water extraction (2:1:0.8, by vol); the sample was extracted overnight and the phases were separated the following day by the addition of chloroform and water (final solvent ratio, 1:1:0.9, by vol, methanol/chloroform/water). The total solvent extract was concentrated (i.e., solvents removed *in vacuo*) using rotary evaporation at 30°C. Lipid class analyses were conducted immediately; for other purposes samples were stored for no more than 3 d in a known volume of chloroform.

Lipids. An aliquot of the total solvent extract was analyzed using an Iatroscan MK V TH10 thin-layer chromatography–flame-ionization detector (TLC–FID) analyzer (Tokyo, Japan) to determine the abundances of individual lipid classes (13). Samples were applied in duplicate or triplicate to silica gel SIII Chromarods (5-μm particle size) using 1 μL disposable micropipets. 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, by vol), a mobile phase resolving nonpolar compounds such as WE, TAG, free fatty acids (FFA), and ST. A second nonpolar solvent system of hexane/diethyl ether (96:4, vol/vol) was also used to separate hydrocarbons from WE and TAG from diacylglycerol. After development, the chromarods were oven-dried and analyzed immediately to minimize adsorption of atmospheric contaminants. The FID was calibrated for each compound class (phosphatidylcholine, cholesterol, cholesteryl ester, oleic acid, squalene, triolein, and DAGE purified from shark liver oil; 0.1–10 μg range). A laboratory standard of WE was used for peak identification, and steryl ester was used for quantification of WE. Based on the TLC–FID analyses and subsequent analysis of component fatty acids and alcohols by gas chromatography (GC), steryl esters were either absent or only present as trace components. WE and steryl esters coelute in the systems used, but results for combined TLC–FID and GC assays indicated they were either absent or only present as trace components. Peaks were quantified on an IBM-compatible computer using DAPA software (Kalamunda, Western Australia). Iatroscan results are generally reproducible to ±10% (13).

Fatty acids and 1-O-alkyl glycerols (GE). An aliquot of the total solvent extract was treated with potassium hydroxide in methanol (5% wt/vol) and saponified under nitrogen for 3 h at 80°C. Nonsaponifiable neutral lipids (e.g., glyceryl ether diols and hydrocarbons) were then extracted into hexane/chloroform (4:1 vol/vol, 3 × 1.5 mL) and transferred to sample vials. Following acidification of the remaining aqueous layer using hydrochloric acid (pH = 2), fatty acids were extracted and methylated to produce their corresponding fatty acid methyl esters using methanol/hydrochloric acid/chloroform (10:1:1, by vol; 80°C, 2 h). Products were then extracted into hexane/chloroform (4:1 vol/vol, 3 × 1.5 mL) and stored at –20°C. The nonsaponifiable neutral lipid fractions were treated with *N,O*-bis-(trimethylsilyl)-trifluoroacetamide (50 μL, 60°C, 1 h) to convert ST to their corresponding trimethylsilyl (TMS) ethers.

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

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

The Iatroscan (TLC–FID) analyzer provides a rapid means of detecting DAGE without any sample treatment and derivatization prior to analysis. To further confirm and elucidate the composition of the DAGE fraction, the process of saponification was used in this study to convert the DAGE to the corresponding GE. The GE are then extracted with other nonsaponifiable neutral lipid material and converted to di-*O*-TMS ether derivatives prior to analysis by GC. GC–MS analysis of the samples readily identified the GE components from their base peak at $m/z = 205$.

Determination of double-bond configuration in fatty acids. Dimethyl disulfide adducts of monounsaturated fatty acids were formed by treating the total fatty acid methyl esters with dimethyl disulfide (14,15). Adducts were then extracted using hexane/chloroform (4:1, vol/vol) and treated with *N,O*-bis-(trimethylsilyl)-trifluoroacetamide to form TMS derivatives prior to GC–MS analysis.

RESULTS

Lipid composition. Clione limacina (order Gymnosomata) has 27.8% DAGE, *S. australis* (order Gymnosomata) has 0.9–1.7% DAGE, and *L. helicina* (order Thecosomata) has only trace amounts of DAGE (Table 1). *Clione limacina* also has over 10 times as much lipid as the other two pteropods (50.8 mg/g, wet weight) (Table 1). The other major lipid classes in *C. limacina* include TAG (22.6%), FFA (23.9%),

TABLE 1
Composition of Lipids from Southern Ocean Pteropods

Sample	Percentage composition[a] (of total lipids)								Lipid (mg/g)[b]
	WE	TAG	FFA	ST	PL	UN	GE	Total	
Clione limacina	0.8	22.6	23.9	2.5	22.1	0.3	27.8	100.0	50.8
Limacina helicina	trace	—	54.9	57.7	39.4	—	trace	100.0	2.4
	0.4	—	59.1	6.3	34.2	—	trace	100.0	6.9
	0.2	—	57.1	6.7	36.0	—	trace	100.0	4.3
Spongiobranchaea australis	0.2	66.7	0.8	0.7	30.0	—	1.7	100.0	3.3
	0.2	39.5	0.4	0.9	58.1	—	0.9	100.0	4.8

[a]Abbreviations: WE, wax ester; TAG, triacylglycerol; FFA, free fatty acid; ST, sterol; PL, phospholipid; UN, unidentified; GE, diacylglyceryl ether.
[b]Wet weight basis.

and phospholipid (PL) (22.1%) (Table 1). *Spongiobranchaea australis* has 39.5–66.7% TAG, 0.4–0.8% FFA, and 30.0–58.1% PL. ST are present in all pteropods (0.7–6.7%) along with minor amounts of WE (trace–0.8%) (Table 1).

ST. The major ST in *C. limacina* include 24-methylcholesta-5,24(28)*E*-dien-3β-ol (24-methylenecholesterol, 21.5%), cholesta-5,22*E*-dien-3β-ol (*trans*-dehydrocholesterol, 19.0%), 24-methylcholesta-5,22*E*-dien-3β-ol (brassicasterol, 17%), cholesta-5,24*E*-dien-3β-ol (desmosterol, 15.6%), cholest-5-en-3β-ol (cholesterol, 11.6%), and 24-norcholesta-5,22*E*-dien-3β-ol (24-nordehydrocholesterol, 6.5%) (Table 2). The major ST in *L. helicina* are similar to those in *C. limacina* and include 24-methylenecholesterol (22.8–24.1%), *trans*-dehydrocholesterol (14–16.4%), brassicasterol (15.8–16.8%), desmosterol (17.4–19.7%), cholesterol (9.1–9.8%), and 24-nordehydrocholesterol (7–9.1%). The sterol composition of *S. australis* differs from both *C. limacina* and *L. helicina* in that *S. australis* has less 24-methylenecholesterol (12.7–12.9%), more *trans*-dehydrocholesterol (30.9–34.0%), slightly more brassicasterol (19.0–20.5%), less desmosterol (4.4–4.5%), slightly less cholesterol (7.7–8.6%), and slightly more 24-nordehydrocholesterol (9.7–9.8%) (Table 2). Five minor ST were also detected: cholesta-5,22*Z*-dien-3β-ol (*cis*-dehydrocholesterol, 1.3–3.2%), 24-ethylcholesta-5,22*E*-dien-3β-ol (stigmasterol, 0.4–1.4%), 24-ethylcholest-5-en-3β-ol (24-ethylcholesterol, 0.2–3.2%), 24-ethylcholesta-5,24(28)*Z*-dien-3β-ol (isofucosterol, 0.9–2.9%), and an unidentified C_{30} ST, containing two double bonds (1.0–2.1%) (Table 2). Five stanols were found in minor amounts in the Southern Ocean pteropods. These include 5α-24-norcholest-22*E*-en-3β-ol (24-nordehydrocholestanol, 0.1–0.6%), 5α-cholest-22*E*-en-3β-ol (dehydrocholestanol, 0.1–0.7%), 5α-cholestan-3β-ol (cholestanol, 0.2–2.2%), 24-methyl-5α-cholest-22*E*-en-3β-ol (brassicastanol, 0.2–0.7%), and 24-methyl-5α-cholest-24(28)*E*-en-3β-ol (24-methylenecholestanol, 0.8–0.9%) (Table 2).

TABLE 2
Sterol Composition of Southern Ocean Pteropods

Sterol	Peak no.[a]	Percentage composition					
		Clione limacina		*Limacina helicina*		*Spongiobranchaea australis*	
24-Nordehydrocholesterol	1	6.52	6.96	9.07	8.58	9.73	9.81
24-Nordehydrocholestanol	2	0.07	—	—	—	0.55	0.58
cis-Dehydrocholesterol	3	1.73	1.65	1.28	1.37	3.19	2.82
trans-Dehydrocholesterol	4	19.05	13.97	16.36	14.90	34.02	30.93
Dehydrocholestanol	5	0.11	—	—	—	0.65	0.65
Cholesterol	6	11.64	9.09	9.79	9.08	8.56	7.72
Cholestanol	7	0.15	0.15	—	—	n.d.[b]	2.16
Desmosterol	8	15.59	19.68	17.42	19.28	4.37	4.49
Brassicasterol	10	17.00	15.82	16.80	15.76	19.03	20.48
Brassicastanol	11	—	0.15	—	—	0.68	0.44
24-Methylenecholesterol	12	21.54	23.61	22.83	24.12	12.93	12.72
24-Methylenecholestanol	13	0.79	0.88	0.75	0.83		
Stigmasterol	14	0.48	1.43	0.44	0.44	0.53	0.36
24-Ethylcholesterol	16	2.20	1.39	1.22	0.20	3.13	3.22
Isofucosterol	18	1.91	2.94	2.54	2.86	0.97	0.87
C_{30}, two double bonds	20	1.01	2.97	1.52	1.75	0.96	1.28
Other		0.22	0.22	—	0.83	0.73	1.4
Total		100.00	100.00	100.00	100.00	100.00	100.00

[a]Peak number on gas chromatogram.
[b]n.d., not determined.

GE. The electron ionization-mass spectra of silylated (1-*O*-alkyl-2,3-di-*O*-TMSi) GE showed the following features: a base peak at 205 *m/z*; ions derived from the glycerol-2,3-di-*O*-TMS moiety, namely, *m/z* 103, 117, 130, 133, and 147; M – 90, M – 104, M – 147, and M – 180. The M^+ was generally not observed. The GC–MS analyses confirmed that the ether lipids of the three Southern Ocean pteropods are a mixture of predominantly saturated with lower levels of monounsaturated 1-*O*-alkyl glyceryl ethers. The principal GE of the three pteropod species is 1-hexa-decylglyceryl ether (16:0); 60% for *C. limacina*, 55% for *S. australis*, and 46% for *L. helicina* (Table 3). The GE 1-penta-decylglyceryl ether (15:0) is second in importance in *C. limacina* (21%), whereas hexadec-9-enylglyceryl ether (16:1) is the second-most abundant GE in *S. australis* (19%), which has only 4% 15:0 GE. *Clione limacina* has less high molecular weight GE (i18:0–20:1 GE summed are 7%), whereas in *S. australis* these GE (i18:0-20:1) are 18% and in *L. helicina* 22% of the total (Table 3). The GE eicosa-9-enylglyceryl ether (20:1) is 8% and 10% in *S. australis* and *L. helicina*, respectively; however in *C. limacina* it is 3% of the total. Also, the GE octadecylglyceryl ether (18:0) is 4% in *S. australis* and *L. helicina* whereas in *C. limacina* it is 1% of the total (Table 3).

Fatty acids. Clione limacina and *L. helicina* both differ substantially from *S. australis* because they have less eicosapentaenoic acid 20:5n-3 (7.2–11.7%) than *S. australis* (21.5–23.3%) 20:5n-3 (Table 4). The sum of the polyunsaturated fatty acids (PUFA) is therefore greater for *S. australis* (46–48%) than for *C. limacina* (26.7%) and *L. helicina* (32.2–42.8%). Docosahexaenoic acid 22:6n-3 is the second-most common PUFA in *S. australis* (18.7–19.1%) and the major PUFA in both *C. limacina* (15.8%) and *L. helicina* (16.3–21.9%). Although levels of 22:5n-3 are generally low, this PUFA is twice as abundant in *L. helicina* (2.6–2.7%) than in *C. limacina* (1.1%) and *S. australis* (1.1–1.4%). The PUFA 20:2n-6 is also more important in *L. helicina* (3.3–3.8%) than *S. australis* (1.1–1.3%), and it was not detected in *C. limacina* (Table 4).

Clione limacina and *L. helicina* also differ substantially from *S. australis* because they have more of the monoene 20:1n-7*c* (11% for *C. limacina*, 17.8–23.1% for *L. helicina*) than *S. australis* (3.7–3.8%) (Table 4). They also have more 20:1n-9*c* (7.7% for *C. limacina* and 3.5–4.6% for *L. helicina*) than *S. australis* (1.7%). The sum of the monounsaturated fatty acids is therefore greater for *C. limacina* (33.4%) and *L. helicina* (26.3–38.8%) than *S. australis* (19.2–19.3%). Despite this, *S. australis* has considerably more 16:1n-7*c* (6.4–6.5%) than *C. limacina* (2.5%) and *L. helicina* (0.6–3.4%). The two isomers of 18:1n-9*c*, and 18:1n-7*c*, together are 8.6% for *C. limacina*, 3.3–10.6% for *L. helicina*, and 5.5–6.4% for *S. australis*.

The principal saturated fatty acids in *C. limacina* and *L. helicina* are palmitic acid (16:0) and stearic acid (18:0) (Table 4). In *C. limacina*, there is 11.8% of 16:0 and 6.6% of 18:0, whereas in *L. helicina* there is 9.8–16.1% of 16:0 and 5.4–16.8% of 18:0. In *S. australis*, the principal saturated fatty acids are 16:0 (17.8–18.2%), 18:0 (3.4–3.8%), and 14:0 (3.7–3.8%). In *C. limacina*, the saturated fatty acids 17:0 (4.8%) and a17:0 (coeluting with 17:1n-8*c*) (8.9%) are also important. Levels of 17:0 and a17:0 in the other pteropods are uniformly low (0.3–2.2%).

TABLE 3
1-*O*-Alkyl Glycerol Composition of Southern Ocean Pteropods

Ether alkyl chain	Percentage composition		
	Clione limacina	*Limacina helicina*	*Spongiobranchaea australis*
14:0	—	8	3
15:0	21	4	4
16:1	3	9	19
16:1	—	4	—
16:0	60	46	55
i17:0	2	—	—
a17:0	3	4	—
17:0	5	3	2
i18:0	<1	—	—
18:1	1	5	6
18:0	1	4	4
i19:0	1	—	—
20:1	trace[a]	3	—
20:1	3	10	8

[a]Trace: less than 0.5%.

DISCUSSION

Occurrence of DAGE in Antarctic zooplankton. The high level of DAGE (28%) in the pteropod *C. limacina* from Antarctic waters (Bransfield Strait) is unusual in marine macrozooplankton (Table 1). Lee (16) reported 19% DAGE in *C. limacina* from Bute Inlet, British Columbia, but the supportive data were unpublished. Published reports on lipid composition of Antarctic invertebrate zooplankton either contain no reference to DAGE or report its presence as less than 1.5%. Hagen (17) found 1.3% DAGE in *C. limacina* collected from the Antarctische Halbinsel, and DAGE was not reported in pooled samples of *C. limacina* collected from the Weddell Sea in 1985. *Limacina helicina* samples had 0–1.5% DAGE (17). From the fatty acid data, it seemed that DAGE constituted 6% in *Clione* specimens (Hagen, W., personal communication). The Thecosome pteropod *Clio pyramidata* had 0.3% DAGE (17). Reinhardt and Van Vleet (5) found TAG, WE, and PL as the major lipid classes in 16 species of Antarctic invertebrate zooplankton, including amphipods, copepods, polychaetes, chaetognaths, cnidarians, tunicates, mysids, and euphausiids as well as mixed plankton. Clarke (18) found 0.3–1.5% DAGE in the Antarctic isopods *Serolis pagenstecheri* and *S. cornuta*. Fricke *et al.* (19) found 0.3–0.6% DAGE in Antarctic krill *Euphausia superba*. Hallgren and Stallberg (20) reported less than 1.5% DAGE in benthic invertebrates including marine crayfish, shrimp, and sea mussels (species identifications not given). Some benthic mollusks, such as *Octopus dofleini*, have 5% DAGE (21). The high DAGE percentage in *C. limacina* is accompanied by a

TABLE 4
Fatty Acid Composition of Southern Ocean Pteropods

Fatty acid	Percentage composition					
	Clione limacina		*Limacina helicina*		*Spongiobranchaea australis*	
14:0	0.51	1.43	1.11	1.38	3.71	3.84
15:0	1.64	—	0.20	0.19	1.00	1.04
16:0	11.75	16.13	12.53	9.79	18.21	17.83
17:0	4.76	0.76	0.37	0.34	1.13	1.18
18:0	6.58	16.75	6.10	5.38	3.43	3.79
19:0	0.44	—	0.18	0.16	0.06	0.07
20:0	0.82	0.80	2.00	0.35	0.07	0.07
22:0	—	—	0.05	—	0.03	0.03
24:0	—	—	0.21	0.08	—	—
Sum saturates	25.68	35.89	21.58	17.66	27.63	27.84
i14:0	—	—	—	—	0.75	0.92
i15:0	—	—	0.13	0.08	0.08	0.08
i16:0	—	—	0.16	0.08	0.11	0.11
i17:0	0.49	0.50	0.52	0.39	0.49	0.47
a17:0[a]	8.92	1.21	1.64	0.96	2.15	1.82
Sum branched	9.41	1.70	2.45	1.52	3.58	3.40
16:1n-9*c*[b]	—	—	—	—	0.14	0.11
16:1n-7*c*	2.49	—	0.58	3.44	6.43	6.47
16:1n-5*c*	—	—	0.11	0.14	0.15	0.13
18:1n-9*c*	3.11	1.92	1.14	6.81	1.57	1.72
18:1n-7*c*	5.53	2.18	2.12	3.75	4.78	4.75
18:1n-7*t*	—	—	—	—	0.04	—
18:1n-5*c*	0.55	—	0.25	0.28	0.40	0.36
20:1n-11 and n-3*c*	2.63	0.89	—	—	—	—
20:1n-9*c*	7.68	3.50	4.56	4.03	1.76	1.73
20:1n-7*c*	10.98	17.75	23.07	19.94	3.68	3.77
22:1n-9*c*	0.25	0.26	0.28	—	0.14	0.13
22:1n-7*c*	0.22	0.10	0.08	—	0.07	0.05
24:1n-9*c*	—	0.04	0.05	—	0.03	0.02
Sum monounsaturates	33.43	26.25	32.23	38.81	19.19	19.25
18:2n-6[c]	1.08	1.45	0.51	10.06	1.60	1.61
18:4n-3	0.35	—	0.41	0.61	0.78	0.66
20:2n-6	—	3.29	3.80	3.36	1.13	1.26
20:3n-6	—	—	—	—	0.25	0.30
20:4n-3	1.24	1.21	1.66	1.37	0.64	0.65
20:5n-3[c]	7.15	7.26	11.74	11.18	21.45	23.29
22:5n-3	1.11	2.63	2.71	2.55	1.41	1.11
22:6n-3	15.81	16.31	21.94	21.15	18.71	19.07
Sum PUFA	26.74	32.15	42.77	41.30	45.98	47.95
Other	4.74	4.00	0.98	0.71	3.62	1.55
Total	100.00	100.00	100.00	100.00	100.00	100.00

[a]Includes 17:1n-8*c*.
[b]The suffixes *c* and *t* denote *cis* and *trans* geometry, respectively, for monounsaturated fatty acids.
[c]Trace amounts of 18:3n-3 and 20:4n-6 were also present, coeluting with 18:2n-6 and 20:5n-3, respectively. Abbreviation: PUFA, polyunsaturated fatty acids.

high lipid content, 50.8 mg/g wet weight, an order of magnitude greater than in *L. helicina* and *S. australis* (Table 1).

Distribution of DAGE in other marine organisms. DAGE is an abundant component of liver oil of certain shark species (22). Plunket shark (*Centroscymnus plunketi*), Pacific sleeper shark (*Somniosus pacificus*) (23), and the leafscale gulper shark (*Centrophorus squamosus*) (24) have livers rich in DAGE. The liver of many of these sharks is over 50% oil; in some it is over 80% (25). The dogfish, *Squalus acanthias*, has 38–45% DAGE in the liver oil, and the holocephalans *Chimaera barbouri*, *C. ogilbye*, and *Hydrolagus colliei* have more than 50% DAGE in the liver oil (3). *Hydrolagus novaezealandiae* has 66% DAGE in its liver oil (26). Methoxyglyceryl ethers, first isolated from Greenland shark liver lipids, have antibiotic activity and inhibit the growth of tumors in mice (27). DAGE is also an abundant component of

certain deep sea squids. The liver and stomach contents of *Moroteuthis robusta* (giant squid, family Onychoteuthidae) and the liver of Gonatid squids (family Gonatidae, *Berryteuthis magister*, *Gonatopsis makko*, and *G. borealis*) also contain significant amounts of DAGE (28–30).

GE composition. The GE composition of three Southern Ocean pteropods (Table 3) is different from deep-sea sharks and squids. Pteropod GE are dominated by 1-hexadecylglyceryl ether (16:0; 46–60%), whereas the major GE in deep-sea sharks and marine DAGE in general is octadec-9-enylglyceryl ether 18:1n-9*c* (42–62%) (23,31). Although deep-sea squid *M. robusta* has less 18:1n-9*c* GE (24–26%) (30), it is much more than the level of 18:1 GE in pteropods (1–6%, Table 3). In addition, the pteropod *C. limacina* has 21% 1-penta-decylglyceryl ether (15:0 GE) which was generally not reported in deep-sea sharks and is only present at 1–1.4% levels in deep-sea squids. At the present time, the reasons for the high relative proportion of the C_{15} GE are not known. Two isomers of 20:1 GE were also observed (Table 3). This observation is similar to the fatty acid profile where two isomers 20:1n-7*c* and 20:1n-9 were also observed. The similarity in profiles is consistent with a common origin. In *C. limacina* in particular, the GE composition is dominated by lower molecular weight GE than in the deep-sea sharks and squids. The GE of *C. limacina* are also different from *L. helicina* and *S. australis* which are characterized by somewhat higher hexadec-7-enylglyceryl ether (16:1n-7*c*; 9–19%) levels than *C. limacina* (3% 16:1n-7*c*) and higher levels of i18:0–20:1 (summed) (22–18%, respectively) (Table 3).

Two isomers of 20:1 were also observed in the alkyl GE diol fraction of *L. helicina*. The double-bond position was not determined for the GE fractions; however, based on the fatty acid profiles, the two alkyl GE chains are presumed to be the n-7 and n-9 isomers. In comparison, only one main isomer was present in *Clione* and *Spongiobranchaea* with trace levels of a second isomer also present; based on retention time data, the main component was presumed to be the 20:1n-7 isomer. This observation is in contrast to the fatty acid profiles where two isomers (20:1n-7*c* and 20:1n-9*c*) were present in all three animals. In addition for *Clione* and *Spongiobranchaea*, the n-7/n-9 ratio for the fatty acids was generally between 1–2. Although the n-7 20:1 isomer was present at higher relative levels than the n-9 isomer in the fatty acid fraction, the ratio of n-7/n-9 for GE was significantly greater than observed for the fatty acid fractions in both *Clione* and *Spongiobranchaea*. We presently can offer no explanation for these ratio differences between lipid classes (GE from DAGE vs. fatty acids from all lipids) in the proportion of isomers. Kang *et al.* (31) have noted similar ratio differences for DAGE GE and total lipid fatty acids from both shark liver oil rich in DAGE fed to Atlantic salmon and for DAGE and total lipid recovered from the salmon feces.

Comparative lipid compositional features. The PL and ST reported in Table 1 probably include membrane structural PL and membrane ST. The relatively higher amounts of PL in *L. helicina* (34.2–39.4%) vs. *C. limacina* (22.1%) are consistent with their lower lipid (2.4–6.9 mg/g wet weight). The same feature is observed for *S. australis* (3.3–4.8 mg/g wet weight). Also *L. helicina* and *S. australis* have trace or low DAGE (Table 1). The high FFA in *L. helicina* (54.9–59.1%) reflect the time necessary for sorting and counting in a heated zooplankton van prior to freezing. The small body size of *L. helicina* compared with *C. limacina* means that lipase enzyme breakdown of TAG (not detectable in *L. helicina*, Table 1) and/or PL occurred more rapidly than in the larger *C. limacina* (23.9% FFA). We have no explanation for the high PL values in *L. helicina* (Table 1). An alternative explanation might be that the FFA are used as an energy store. The high TAG (39.5–66.7%) in *S. australis* reflect a lack of lipase activity because *S. australis* was sorted and counted in a laboratory aboard the R/V *Aurora Australis* maintained at −1°C. Freezing was also accomplished more rapidly after capture and was in liquid nitrogen. The presence of WE (trace to 0.8%) in all pteropods (Table 1) may mean that these WE were obtained from the food chain. Copepods are rich sources of WE in polar waters (1) and may be the dietary source of WE in pteropods.

The similar ST composition of *C. limacina* and *L. helicina* (Table 2) reflects the feeding selectivity of *C. limacina*, which is believed to feed exclusively on *L. helicina* (9). The ST of *S. australis* differ from *L. helicina* and *C. limacina* (i.e., less 24-methylenecholesterol and more *trans*-dehydrocholesterol, Table 2) because *S. australis* feeds exclusively on certain *Clio* species, such as *C. pyramidata* (17). Both *Limacina* and *Clio* are herbivorous Thecosome pteropods, and their ST are derived from dietary phytoplankton. Desmosterol, for example, in *L. helicina* (17.42–19.68%, Table 3) is probably derived from dealkylation of dietary phytosterols. Desmosterol may also be of some importance in providing cholesterol, since it is an intermediate in cholesterol synthesis. Gastropods can synthesize cholesterol *de novo* (32), but it is a very slow process, as evidenced by the low cholesterol levels in these pteropods (11.6% cholesterol in *C. limacina*, Table 2). Cholesterol was present in only six of 14 diatom species studied by Barrett *et al.* (33). The ST 24-methylenecholesterol (21.5–24.1% in *C. limacina* and *L. helicina*) is the main ST in the diatom *Chaetoceros* (34). Brassicasterol is a major ST in some prymnesiophytes such as *Phaeocystis*, and it is important in *L. helicina* (15.8–16.8%, Table 2). The phytoplankton composition of the Bransfield Strait waters where *C. limacina* and *L. helicina* were collected was characterized by microplanktonic diatoms, such as *Chaetoceros*, *Nitzschia*, and *Rhizoselenia*, as well as 2–5-nm flagellates during 1990–1993 (35). *Rhizoselenia setigera* was reported to have 94.2% desmosterol and *Nitzschia closterium* 100% desmosterol (33). The area where *S. australis* was collected is characterized by a different phytoplankton assemblage, which may be reflected in its different ST composition compared to that in the other two pteropods (Table 2).

The similar fatty acid composition of *C. limacina* and *L. helicina* bears out the feeding selectivity already discussed. Despite this, there are differences in the percentage composi-

tion of a17:0/17:1n-8c which are unexplained (Table 4). *Spongiobranchaea australis* fatty acids are different from those of *C. limacina* and *L. helicina* in that *S. australis* has over twice as much eicosapentaenoic acid 20:5n-3 (Table 4). This may indicate greater availability of this n-3 PUFA in the phytoplankton grazed by *Clio pyramidata*, the food of *S. australis*. The 20:5n-3 PUFA is found in the diatoms *Chaetoceros calcitrans*, *Skeletonema costatum*, and *Thallassiosira pseudonana*, where it is 4.6–11.1% of the total fatty acids (36). *Clione limacina* and *L. helicina* are enriched in the monoenes 20:1n-7c and 20:1n-9c in contrast to *S. australis*. This also emphasizes the different sources of food available to these two groups of pteropods.

Potential uses of DAGE. DAGE may be important in the treatment of hematopoiesis and radiation sickness (37,38). Glyceryl ethers are useful as surfactants in cosmetics and ointments, and ratfish liver DAGE are intermediates in the synthesis of alkylacetoglycerophosphocholine, a platelet-activating factor (29,39). Methoxy-glyceryl ethers have been reported in shark liver oil (20) and have antibiotic activity and inhibit the growth of tumors in mice (27). It has been stated that the glyceryl ethers, including those of chimyl or batyl alcohol (or their monounsaturated derivatives), have enormous economic potential because they possess this range of healing activity (40). Shark ether lipids can become a good source of ether-linked lipids, which increase membrane plasmalogen levels in patients suffering from the genetic Zellwegere cerebrohepatorenal syndrome (41). It would be interesting to confirm that the pteropod DAGE also have anticarcinogenic activity, since pteropod culture could be a rapid and renewable source of DAGE. In contrast, the presently utilized deep-sea shark and ratfish may be showing signs of overfishing in selected locations. Harvest of pteropods therefore might be an alternative source of DAGE. *Clione limacina* amounts to 80% of the total Gymnosome population in the north Atlantic Ocean and is so common that it is a food of the Greenland Right whale and some fishes (9). It reaches 50–70 mm in length in cold waters. Further analyses of *C. limacina* and other pteropods from a range of locations are recommended and will be pursued. The economics for harvesting *Clione* presently may appear unattractive. However, culture of this species, including as a possible direct source of DAGE or for use as a source of transgenic material for further application, may have better potential.

A possible buoyancy role for DAGE. A convincing argument for buoyancy regulation as a function for DAGE was made by Malins and Barone (42), who found the concentration ratios of DAGE to TAG to significantly increase in livers of weighted *Squalus acanthias* when compared to an unweighted control group. DAGE gives 23% more lift than TAG in 1.025 g/cc sea water (4). The change was not due to selective mobilization of TAG but involved a net increase in DAGE. Malins and Barone (42) suggested that *S. acanthias* regulates buoyancy by the selective metabolism of DAGE and TAG during vertical migrations, and that this may substitute for the swimbladder in teleost fishes that is not found in sharks.

We propose a buoyancy function for the 28% DAGE found in *C. limacina*. Most lipids are less dense than seawater, and therefore increase the overall buoyancy of aquatic organisms, but their main functions are energy storage and as structural (membrane) components (43). Lee (1), for example, starved *Clione* from Canadian waters for 3 mon and observed a loss of 85% of their lipid (mostly TAG). Significant structural differences between *Clione* and *Limacina*, however, support a buoyancy function. *Limacina* is a Thecosome pteropod, with a calcareous test (shell), but the density of the test is offset by bubbles around it. *Limacina* also has long floppy swimming appendages, like *Clio pyramidata*, another Thecosome (shelled) pteropod (44). These swimming appendages help these pteropods maintain their position in the water column, and the increased surface area slows down sinking. In contrast, both *C. limacina* and *S. australis* are Gymnosome (naked) pteropods, lack bubbles, and have compact, hard bodies, with short, stubby swimming appendages. Both *Clione* and *Spongiobranchaea* have DAGE (28% and 0.9–1.7%, respectively, Table 1), whereas *Limacina* has only trace amounts of DAGE. *Clione* is lipid-rich, whereas *Limacina* is low in lipid (17) (Table 1). Since *S. australis* has both low lipid and low DAGE, it may use an alternative strategy for buoyancy. The Gymnosome pteropods selectively feed on the herbivores *Limacina* and *Clio*, so it is certainly to their advantage to remain at the same level in the water column as their prey. DAGE may give just the added uplift to accomplish the appropriate buoyancy for this selective feeding in *C. limacina*.

ACKNOWLEDGMENTS

This work was supported in part by funding from FRDC. We thank J. Martin and R. Hewitt for the Antarctic Marine Life Research Expedition to the Elephant Island study area and the Australian National Antarctic Research Expedition for collection of samples in East Antarctica. V. Siegal, D. Outram, and V. Loeb gave invaluable help in collection and identification of samples. Thanks to V. Loeb for sharing her insights into pteropod biology. C. Phleger thanks the Department of Biology, San Diego State University, for sabbatical leave time to do research. Professor R.G. Ackman and an anonymus referee provided constructive comments to the manuscript.

REFERENCES

1. Lee, R.F., and Patton, J.S. (1989) Alcohol and Waxes, in *Marine Biogenic Lipids, Fats and Oils* (Ackman, R.G., ed.), Vol. 1, pp. 73–102, CRC Press, Boca Raton.
2. Benson, A.A., and Lee, R.F. (1975) The Role of Wax in Oceanic Food Chains, *Sci. Am. 232*, 77–86.
3. Sargent, J.R. (1989) Ether-Linked Glycerides in Marine Animals, in *Marine Biogenic Lipids, Fats, and Oils* (Ackman, R.G., ed.) Vol. 1, pp. 175–197, CRC Press, Boca Raton.
4. Sargent, J.R. (1976) The Structure, Metabolism, and Function of Lipids in Marine Organisms, in *Biochemical and Biophysical Perspectives in Marine Biology* (Malins, D.C., and Sargent, J.R., eds.) Vol. III, pp. 149–212, Academic Press, London.
5. Reinhardt, S.B., and Van Vleet, E.S. (1984) Lipid Composition of Antarctic Midwater Invertebrates, *Antarctic J. 1984 Review*, 139–141.

6. Sargent, J.R., Parkes, R.J., Mueller-Harvey, I., and Henderson, R.J. (1987) Lipid Biomarkers in Marine Ecology, in *Microbes in the Sea* (Sleigh, M.A., ed.) pp. 119–138, Ellis Horwood, Chichester.
7. Quetin, L.B., and Ross, R.M. (1991) Behavioral and Physiological Characteristics of the Antarctic Krill, *Euphausia superba, Amer. Zool. 31*, 49–63.
8. Nichols, P.D., Skerratt, J.H., Davidson, A., Burton, H., and McMeekin, T.A. (1991) Lipids of Cultured *Phaeocystis pouchetii:* Signatures for Food-web, Biogeochemical and Environmental Studies in Antarctica and the Southern Ocean, *Phytochemistry 30*, 3209–3214.
9. Raymont, J.E.G. (1983) *Plankton and Productivity in the Oceans*, Vol. 2, Zooplankton, 2nd edn., pp. 146–148, Pergamon Press, Frankfurt.
10. Martin, J. (1996) AMLR 1995/96 Field Season Report LJ-95. 14, Southwest Fisheries Science Center, Antarctic Ecosystem Research Group, La Jolla, CA.
11. Hewitt, R.P., and Demer, D.A. (1991) Krill Abundance, *Nature 353*, 310.
12. Bligh, E.G., and Dyer, W. (1959) A Rapid Method of Total Lipid Extraction and Purification, *Can. J. Biochem. Physiol 37*, 911–917.
13. 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 Environmental Samples, *J. Planar Chromatogr. 4*, 19–26.
14. Dunkelblum, E., Tan, S.H., and Silk, R.J. (1985) Double-Bond Location in Monounsaturated Fatty Acids by Dimethyl Disulphide Derivitization and Mass Spectrometry, *J. Chem. Ecol. 11*, 265–277.
15. Nichols, P.D., Guckert, J.B., and White, D.C. (1986) Determination of Monounsaturated Fatty Acid Double-Bond Position and Geometry for Microbial Monocultures and Complex Consortia by Capillary GC–MS of Their Dimethyl Disulphide Adducts, *J. Microbiol. Meth. 5*, 49–55.
16. Lee, R.F. (1974) Lipids of Zooplankton from Bute Inlet, British Columbia, *J. Fish. Res. Bd. Can. 31*, 1577–1588.
17. Hagen, W. (1988) On the Significance of Lipids in Antarctic Zooplankton, *Ber. Polarforsch. 49*, 1–117.
18. Clarke, A. (1984) Lipid Composition of Two Species of *Serolis* (Crustacea: Isopoda) from Antarctica, *Br. Antarct. Surv. Bull. 64*, 37–53.
19. Fricke, H., Gerken, G., and Oehlenschlager, J. (1986) 1-*O*-Alkylglycerolipids in Antarctic krill (*Euphausia superba* Dana), *Comp. Biochem. Physiol. 85B*, 131–134.
20. Hallgren, B., and Stallberg, G. (1978) Occurrence, Synthesis, and Biological Effects of Substituted Glyceryl Ethers, *Prog. Chem. Fats Other Lipids 16*, 45–58.
21. Mangold, H.K. (1983) Ether Lipids in the Diet of Humans and Animals, in *Ether Lipids. Biochemical and Biomedical Aspects* (Mangold, H.K., and Poltauf, F., eds.) pp. 231–237, Academic Press, New York.
22. Kayama, M., Tsuchiya, Y., and Nevenzel, J.C. (1971) The Glyceryl Ethers of Some Shark Liver Oils, *Nippon Suisan Gakkaishi 37*, 111–118.
23. Bakes, M.J., and Nichols, P.D. (1995) Lipid, Fatty Acid, and Squalene Composition of Liver Oil from Six Species of Deep-Sea Sharks Collected in Southern Australian Waters, *Comp. Biochem. Physiol. 110B*, 267–275.
24. Deprez, P.P., Volkman, J.K., and Davenport, S.R. (1990) Squalene Content and Neutral Lipid Composition of Livers from Deep-Sea Sharks Caught in Tasmanian Waters, *Aust. J. Mar. Freshwater Res. 41*, 375–387.
25. Davenport, S.R., and Deprez, P.P. (1989) Market Opportunities for Shark Liver Oil, *Aust. Fish* (November), 8–10.
26. Hayashi, K., and Takagi, T. (1980) Composition of Diacylglyceryl Ethers in the Liver Lipids of Ratfish, *Hydrolagus novaezealandiae, Nippon Suisan Gakkaishi 46*, 855–861.
27. Hayashi, K., and Takagi, T. (1982) Characteristics of Methoxyglyceryl Ethers from Some Cartilaginous Fish Liver Lipids, *Nippon Suisan Gakkaishi 48*, 1345–1351.
28. Hayashi, K. (1989) Occurrence of Diacyl Glyceryl Ethers in Liver Lipids of Gonatid Squid, *Gonatopsis borealis, Nippon Suisan Gakkaishi 55*, 1383–1387.
29. Hayashi, K., and Kawasaki, K. (1985) Unusual Occurrence of Diacylglyceryl Ethers in Liver Lipids from Two Species of Gonatid Squids, *Nippon Suisan Gakkaishi 51*, 593–597.
30. Hayashi, K., Kishimura, H., and Sakurai, Y. (1990) Level and Composition of Diacylglyceryl Ethers in Different Tissues and Stomach Contents of Giant Squid *Moroteuthis robusta, Nippon Suisan Gakkaishi 56*, 1635–1639.
31. Kang, S.-J., Lall, S.P., and Ackman, R.G. (1997) Digestion of the 1-*O*-Alky Diaclyglycerol Ethers of Atlantic Dogfish Liver Oils by Atlantic Salmon, *Salmo salar, Lipids 32*, 19–30.
32. Goad, L.J. (1978) The Sterols of Marine Invertebrates: Composition, Biosynthesis, and Metabolites, in *Marine Natural Products: Chemical and Biological Perspectives* (Scheuer, P.J., ed.), pp. 45–77, Academic Press, New York.
33. Barrett, S.M., Volkman, J.K., and Dunstan, G.A. (1995) Sterols of 14 Species of Marine Diatoms (Bacillariophyta), *J. Phycol. 31*, 360–369.
34. Tsitsa-Tzardis, E., Pattersor G.W., Wikfors, G.H., Gladu, P.K., and Harrison, D. (1993) Sterols of *Chaetoceros* and *Skeletonema, Lipids 28*, 465–467.
35. Villafane, V.E., Helbling, E.W., and Holm-Hansen, O. (1995) Spatial and Temporal Variability of Phytoplankton Biomass and Taxonomic Composition Around Elephant Island, Antarctica, During the Summers of 1990–1993, *Mar. Biol. 123*, 677–686.
36. Volkman, J.K., Jeffrey, S.W., Nichols, P.D., Rogers, G.I., and Garland, C.D. (1989) Fatty Acid and Lipid Composition of 10 Species of Microalgae Used in Mariculture, *J. Exp. Mar. Biol. Ecol. 128*, 219–240.
37. Blomstrand, R., and Ahrens, E. (1959) Absorption of Chimyl Alcohol in Man, *Proc. Soc. Exp. Biol. Med. 100*, 802–805.
38. Nichols, P.D., Nichols, D.S., and Volkman, J.K. (1993) Recent Developments with Marine Oil Products in Australia, *Chem. Aust. 60*, 336–340.
39. Muramatsu, T., Totani, N., and Mangold, H.K. (1981) A Facile Method for the Preparation of 1-*O*-Alkyl-2-*O*-acetoyl-*sn*-glycero-3-phosphocholines (Platelet Activating Factor), *Chem. Phys. Lipids 29*, 121–127.
40. Bordier, C.G., Sellier, N., Foucault, A.P., and LeGoffic, F. (1996) Purification and Characterization of Deep-Sea Shark *Centrophoros squamosus* Liver Oil 1-*O*-Alkylglycerol Ether Lipids, *Lipids 31*, 521–528.
41. Jeong, B., Ohshima, T., Ushio, H., and Koizumi, C. (1996) Lipids of Cartilaginous Fish: Composition of Ether and Ester Glycerophospholipids in the Muscle of Four Species of Shark, *Comp. Biochem. Physiol. 113B*, 305–312.
42. Malins, D.C., and Barone, A. (1970) Glyceryl Ether Metabolism: Regulation of Buoyancy in Dogfish, *Squalus acanthias, Science 167*, 79–80.
43. Phleger, C.F. (1991) Biochemical Aspects of Buoyancy in Fishes, in *Biochemistry and Molecular Biology of Fishes* (Hochachka, P.W., and Mommsen, T.P., eds.), Chap. 9, pp. 209–247, Elsevier Science Publishers, Amsterdam.
44. Barnes, R.D. (1980) *Invertebrate Zoology*, 4th edn., Saunders College, Philadelphia.

[Received November 19, 1996, and in final revised form April 15, 1997; revision accepted May 22, 1997]