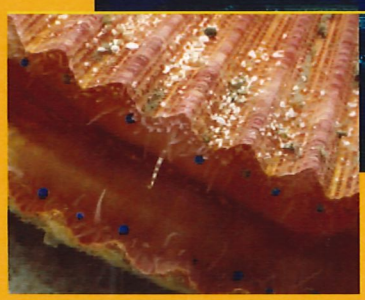
Nutritional Value

OF AUSTRALIAN SEAFOOD II



Factors affecting oil composition of edible species

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FRDC Project 1999/331

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Nutritional Value of Australian Seafood II. Factors Affecting Oil Composition of Edible Species

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1. NON-TECHNICAL SUMMARY

1999/331 Nutritional Value of Australian seafood II. Factors Affecting Oil Composition of Edible Species

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OBJECTIVES

- Expand the current database on the nutritional (oil) composition of principal Australian seafood, including additional industry requested species, and examine seasonal and spatial differences, variation between aquacultured and wild-caught specimens and processing effects (cooking).

- Publish the results in suitable format(s) for use by various fisheries associated marketing agencies, and medical and consumer groups.

OUTCOMES ACHIEVED

A comprehensive Guide – "*Seafood the Good Food II*" has been prepared and used to communicate the health benefits of both wild and farmed Australian seafood to the seafood industry, nutritionists and consumer groups.
The project team has worked directly with the Australian industry to examine further species of seafood. Cooking and other value-adding have no discernable effects on the omega-3 PUFA in seafood. Aquafeeds can be tailored to optimize omega-3 PUFA content and composition in cultured (farmed) seafood.

The content and composition of the oil from 79 species of Australian fish, shellfish and crustaceans were examined. The nutritional value of Australian species was determined with respect to oil composition, as was how oil content may differ with aquaculture and processing, including cooking and other value-adding. This knowledge has been transferred, in various formats (e.g. Guide, reports to selected clients, poster, flier), to the Fishing Industry, nutritionists and other consumer groups to better utilize the total catch, including waste products.

In seafood, oils are the second largest component after protein. Oils have a variety of important roles; they serve as concentrated stores of energy, as fuel molecules and as components of membranes. Of main importance are triglycerides, polar lipids and cholesterol. Triglycerides serve as an energy store and polar lipids and cholesterol are structural components of cell membranes. Cholesterol, when in dietary excess, may be a factor in coronary heart disease and other disorders. The main components of the oils are saturated and unsaturated fatty acids. EPA [eicosapentaenoic acid, 20:5(n-3)] and DHA [docosahexaenoic acid, 22:6(n-3)] are long-chain omega-3 polyunsaturated fatty acids (LC

omega-3 PUFA) essential in the human diet. The omega-3 PUFA are largely obtained through the diet since humans generally are unable to synthesize them. AA [arachidonic acid, 20:4(n-6)] is an omega-6 PUFA which is a precursor of prostaglandins (which modulate hormone activity) and other eicosanoids (C_{20} physiologically active compounds).

The marine-derived omega-3 PUFA, in particular EPA and DHA, have a wide range of potential health benefits, particularly with respect to the prevention of coronary heart disease and rheumatoid arthritis. They also may play a role against some forms of cancer and other disorders, although further research and trials are required. Omega-3 PUFA may be also beneficial for infant brain and retina function and development. The guide for seafood marketers "*What's so healthy about seafood*?" provides further information.

Communication of results was achieved through the launch of the guide *Seafood the Good Food II* in Hobart in May 2002. The guide provides complete oil profiles for all species analysed during project 1999/331, and is available from CSIRO Publishing. In addition, a "Good Oils" poster, flier, and factsheet, were also released at the May 2002 launch for national distribution. The national launch, printing and distribution of the promotional material was sponsored by the Fisheries Research and Development Corporation and the NSW Master Fish Merchants Association. Other media releases, conference presentations and scientific reports occurred during the project.

For the Australian species analysed during the "Good Oil" studies the summary findings included:

- Relative to other food groups, wild-caught, cultured and value-added seafood are the best and most readily available source of EPA and DHA;
- Most Australian fish have high levels of omega-3 PUFA (average 235 mg/100 g, range 13 to 3760 mg/100 g) and low levels of cholesterol (average 28 mg/100 g);
- Prawns have lower levels of omega-3 PUFA (average 130 mg/100 g) and higher levels of cholesterol (average 130 mg/100 g) than fish;
- Australian fish generally have higher relative levels of DHA than fishes from the Northern Hemisphere;
- Fish from warmer waters generally have lower omega-3 / omega-6 ratios than fish from temperate waters, due largely to higher relative levels of AA;
- Fish generally contain polar oil and/or triglyceride, although a few species contain unusually high contents of wax ester, hydrocarbon or diacylglyceryl ether;
- Cultured (farmed) seafood was generally an excellent source of omega-3 PUFA, and the oil in the feed can be manipulated to increase oil levels in products;
- Cooking and processing have no discernable effect on the content and composition of the omega-3 PUFA in seafood; and
- Some variation was observed with season and location for selected fish and shellfish, but the differences generally had little effect on oil quality.

The effect of aquaculture varied between species. Cultured finfish such as barramundi, striped trumpeter, Tasmanian Atlantic salmon and other species contained highly elevated concentrations of LC-omega-3 PUFA using current feeding practices. In contrast, farmed banana prawns contained lower LC-omega-3 PUFA content, in particular DHA, relative to wild specimens.

Cooking trials were performed using a range of methods (e.g. grilling, pan and deep frying, microwave); LC-omega-3 PUFA content was not adversely affected by cooking. By-product (e.g. skin, frame, head, trim, gut) from seafood processing also contained elevated LC-omega-3 PUFA content, and therefore represents an excellent source of material for consideration of value-adding.

Our findings, together with earlier results from FRDC project 1995/122 (Nichols *et al.* 1998 a&b), and earlier Australian reports, provide oil compositional information for use in the selection of fish or fish oils from various species for nutritional studies, and for the marketing and communication of the health benefits of seafood. Standardised procedures have been adopted throughout this study, however, intra-species variation may occur. The results from this study serve therefore as a guide for medical practitioners, nutritionists and other user groups. The fact that intra-species or other forms of variation (e.g. seasonal or regional) may occur should be noted, although the differences were generally not large enough to greatly influence oil quality for consumers.

It is concluded that seafood is the best source of LC omega-3 PUFA from the common food groups. Further comparative nutritional studies where different fish and/or marine oils from various species are used will provide additional information on more specific benefits of LC omega-3 PUFA.

KEYWORDS: Australian seafood, marine oils, omega-3 polyunsaturated fatty acids (PUFA), DHA, EPA

2. ABBREVIATIONS

AA	Arachidonic acid, 20:4ω6, also termed 20:4(n-6)
AAOCS	Australasian Section of the American Oil Chemists' Society
CAAB	Code for Australian Aquatic Biota used in fisheries data analysis (www.marine.csiro.au/caab)
CHD	Coronary heart disease
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DAGE	Diacylglyceryl ether
DHA	Docosahexaenoic acid, 22:6ω3, also termed 22:6(n-3)
DPA	Docosapentaenoic acid, 22:5ω3, also termed 22:5(n-3)
EPA	Eicosapentaenoic acid, 20:5ω3, also termed 20:5(n-3)
FAME	Fatty acid methyl ester
FFA	Free fatty acid
FID	Flame ionization detector
FRDC	Fisheries Research and Development Corporation
GC	Gas chromatography
GC-MS	Gas chromatography-mass spectrometry
HC	Hydrocarbon
IS	Internal standard
LA	Linoleic acid, 18:2ω6, also termed 18:2(n-6)
LC-PUFA	Long-chain ($\geq C_{20}$) (see also PUFA)
MUFA	Monounsaturated fatty acids containing one carbon-carbon olefinic centre, generally with *cis* configuration. e.g., oleic acid [18:1ω9c, also termed 18:1(n-9)c]
Omega-3	Also termed (n-3) or ω3. Family of polyunsaturated acids with two or more *cis*-unsaturated centres, separated from each other by one methylene group and having the first unsaturated centre three carbons from the end methyl
Omega-6	Also termed (n-6) or ω6. Family of polyunsaturated acids with two or more *cis*-unsaturated centres, separated from each other by one methylene group and having the first unsaturated centre six carbons from the end methyl
PL	Polar lipid (includes mostly phospholipid)
PUFA	Polyunsaturated fatty acids
SAT	Also termed SFA. Saturated fatty acids without carbon-carbon unsaturation, e.g., myristic (14:0) and palmitic (16:0) acids
ST	Sterol
TAG	Triacylglycerol, also termed triglyceride
TLC-FID	Thin layer chromatography-flame ionization detection
TMS	Trimethyl silyl (ether derivative)
TSE	Total solvent extract
UN	Unidentified
WE	Wax ester

3. BACKGROUND

The Australian seafood industry is a billion dollar business; the current market value approaches $1.5B (FRDC). Marketing of seafood is a vital but difficult component of the industry. In Australia, consumption of seafood is still low compared to other food products such as beef, lamb and chicken. The marketing of fish products relies on many factors, including the beneficial health aspects of seafood. Marketing of seafood worldwide has in recent years utilised the fact that most fish contain oils with high levels of nutritionally important omega-3 polyunsaturated fatty acids (PUFA). This fact is considered by nutritionists and medical authorities to be an attractive and beneficial feature for the increased intake of seafood in our diet.

The main components of the oils are saturated and unsaturated fatty acids. EPA [eicosapentaenoic acid, 20:5(n-3)] and DHA [docosahexaenoic acid, 22:6(n-3)] are long-chain omega-3 polyunsaturated fatty acids (LC omega-3 PUFA) essential in the human diet. Omega-3 PUFA are largely obtained in our diet since humans generally are unable to synthesize them.

EPA and DHA have been demonstrated to be beneficial against various human disorders (Kinsella 1986 & 1987). It is now accepted that fish-derived PUFA decrease the incidence of coronary heart disease (CHD), with experimental studies demonstrating specific benefits in preventing fatal disorders of heart rhythm and blood lipid disorders (e.g. McLennan *et al.* 1988 & 1992; Suzukawa *et al.* 1995). The omega-3 PUFA confer beneficial effects on cardiovascular health through several mechanisms. Compared to saturated fatty acids, which result in increased serum cholesterol levels, the omega-3 PUFA do not affect plasma cholesterol levels. Acute coronary disease is usually caused by thrombosis formation or an episode of malignant cardiac arrhythmia. Antithrombogenic effects may be achieved by increasing the amount of fish-derived omega-3 fatty acids in the diet. Experimental studies in animals have shown that substitution of omega-6, as well as omega-3, fatty acids for saturated fatty acids in the diet reduces the frequency of cardiac arrhythmia with greatest benefit from omega-3 PUFA (e.g. McLennan *et al.* 1992 & 1993). From this evidence, eating fish two or three times per week appears to result in measurable benefits with respect to parameters examined in CHD studies, especially in prevention of primary fatal cardiac arrhythmia (Siscovick *et al.* 1995). The guide for seafood marketers "*What's so healthy about seafood*?" provides further information on the health benefits of seafood.

Omega-3 PUFA also may play a role in the treatment of inflammatory diseases such as rheumatoid arthritis (Cleland and James 1997) and in infant nutrition (Carson 1995; Connor 1997; Heird *et al.* 1997). Similarly, omega-3 PUFA may play a role against hypertension (Howe and Nestel 1992, Rayner and Howe 1995). The biochemical effects of plant and fish-derived oils in some forms of cancer therapy have been examined (e.g. Burns and Spector 1994; Horrobin 1997), although further research and trials are required.

Prior to the recent introduction and increased usage of canola oil, the Australian diet generally contained only low levels of omega-3 PUFA (canola oil contains monounsaturated fatty acids in addition to the C_{18} omega-3 PUFA). The National Health and Medical Research Council recommends that the level of omega-3 PUFA should be increased in the diet (NHMRC 1991; Howe 1998), in line with scientific evidence described above. Common practical advice for

most people is to eat more seafood containing omega-3 PUFA. An aim of our marine oils research has been therefore to provide practical nutritional information on many Australian types of seafood.

In the recently completed and nationally promoted FRDC project 1995/122, we examined the content and composition of the oil from nearly 200 species of Australian fish, shellfish and crustaceans (see FRDC Final Report and *Seafood the Good Food*). The nutritional value of Australian species was determined with respect to oil composition.

For the Australian species analysed during project 1995/122 the findings included:

- Local fish generally had lower levels of oil than species from northern hemisphere waters
- Most fish have high levels of omega-3 PUFA and low levels of cholesterol.
- Australian seafood contain attractive levels of omega-3 PUFA, typically tenfold or more greater than other food groups
- The relative level of PUFA in fish generally increased with decreasing oil content, suggesting that better oil quality occurs for the low oil content species, and that Australian fish are an excellent source of the essential omega-3 PUFA
- Prawns have lower levels of omega-3 PUFA and higher levels of cholesterol than fish.
- Australian fish generally have higher relative levels of DHA compared with fish, and nutritional supplements containing fish oil, from northern hemisphere waters
- Fishes from warmer waters and those with specific diets have lower omega-3 / omega-6 ratios compared with fishes from temperate waters, due largely to higher relative levels of arachidonic acid (AA)
- Season, diet and other factors can influence the oil and fatty acid content and composition of seafood.

Our findings provided oil compositional information for use in the selection of fish or fish oils from various species for human nutritional studies, and for the marketing and communication of the health benefits of seafood. The results from the study serve as a guide for medical practitioners, nutritionists and other user groups. It was concluded that seafood is clearly the best source of LC omega-3 PUFA from the common food groups. Further nutritional studies where different fish and/or marine oils are used will provide additional information on the benefits of LC omega-3 PUFA.

With this background, the FRDC-funded Fish Oil study (1995/122) was completed, including a national launch in Sydney in August 1998 of the *Seafood the Good Food* Guide. Prior to and following the launch of results for the project, including the Guide, further interest was received to build on the project outcomes. Following consultation with a wide range of researchers and State and Federal Agencies, a series of recommendations for further research were included in the final FRDC final report (1995/122). These included: examination of additional species; the effect of season; the spatial and temporal variation in oil composition; examination of other tissues; differences between aquaculture versus wild fisheries; the effect of processing (e.g. cooking). The project was linked with the Industry Extension project (1996/340) and Martin Bowerman of Shaun Somerset and provided results for incorporation in the guide produced for seafood marketers "*What's so healthy about seafood*?" As with FRDC project 1995/122, we aimed to extend the strong association established with NSW MFMA and other state bodies. The results of project 99/331 have been reported in two forms:

1. The Guide *Seafood the Good Food II* (Mooney et al. 2002). Results in the Guide are presented in three Chapters:
   Chapter 2 – oil composition of cultured (farmed) seafood
   Chapter 3 – effects of processing and other factors on oil composition of seafood
   Chapter 4 – the oil composition of Australian seafood.

2. Final report FRDC 1999/331. This includes chapter 7 with a summary of chapter 4 from the Guide, followed by chapters 8 and 9 detailing the oil composition of escolar and rudderfish containing unusually high levels of non-saponifiable lipids. Chapters 10-22 detail outcomes for specific projects performed in partnership with industry and other collaborators examining aspects of the effects of aquaculture, processing and other factors on oil content and composition. The format used for individual chapters varies, and generally follows that used in scientific manuscripts and reports prepared for collaborators and clients to project 1999/331.

4. NEED

Following the successful completion and launch by FRDC and CSIRO of *Seafood the Good Food* in 1998, considerable feedback indicated the need for follow up research. Industry needs communicated included:

1. The need for examination of additional wild-caught species for various client groups. Liaison with state industry councils indicated an additional 80 species for analysis.
2. Seasonal differences. The effect of season on oil composition of seafood was examined for 4 species in project 1995/122, and needed to be examined for further target species.
3. Spatial variation in oil composition of commercial fish and other seafood needs to be further examined.
4. Other tissues needed to be examined. In project 1995/122 we examined fish after the skin (and associated subcutaneous fat) was removed. Therefore results for oil and PUFA content are conservative for some species. Higher amounts of omega-3 PUFA content may be obtained through consumption of whole fish, including the skin. Examination of omega-3 PUFA levels in other fish tissues therefore was needed for selected species.
5. Examination of the differences between aquaculture versus wild fisheries. Aquaculture is predicted to supply a larger source of seafood to the Australian domestic market, therefore research and development on alternative non-fish based feeds is underway. Care must be taken to ensure that the health benefits of seafood, in particular the omega-3 PUFA are not compromised. Research was needed to therefore compare aquaculture versus wild-caught individuals of selected species.
6. Processing. Examination of the effect of cooking methods and other factors on oil and PUFA content and composition was required.

5. OBJECTIVES

The project objectives were unchanged from those submitted in the original proposal.

1. Expand the current database on the nutritional (oil) composition of principal Australian seafood, including additional industry requested species, and examine seasonal and spatial differences, variation between aquacultured and wild-caught specimens and processing effects (cooking).

2. Publish the results in suitable format(s) for use by various fisheries associated marketing agencies, and medical and consumer groups.

6. METHODS

6.1 Samples

The majority of muscle (flesh) samples analysed were subsamples from collections made for the *Handbook of Australian Seafood - A Guide to Whole Fish and Fillets.*

A survey of State Advisory bodies and other organisations was conducted. These organisations indicated priority species or species of potential importance to the Australian seafood industry. The final list of priority species to be analysed during Project 1999/331 was completed in late 1999.

Following announcement of the commencement of the project, further requests for analyses of additional species were received from other companies and organisations. While there was some duplication between the State's priority species and these other requests, the final list of species analysed covers 79 species (58 fish, 9 cructaceans, 9 shellfish, 3 other invertebrates).

Specimens were obtained from a variety of sources including commercial fishers, aquaculture farms, seafood markets and research cruises. The sampling and preservation logistics for the Handbook study provided a unique opportunity to obtain high integrity specimens with confident identification of the samples that was vitally important to the success of this study.

Most muscle samples were taken immediately after capture or after purchase from the market. Some samples were taken from individual specimens which were frozen after capture and transported to Hobart. For most species 5-10 individual specimens were available, from which three were randomly selected for analysis. In a few cases, only 1 or 2 individuals were analysed as further samples were not available. All fish muscle samples were taken from the right "shoulder" region, an area normally included in a fillet. Samples were of white meat. Skin and subcutaneous fat were excluded from muscle samples. Samples were taken from the tail of prawns and lobsters, legs of crabs and either whole body or abductor muscle of shellfish. All tissues were stored at -80°C until analysed.

6.2 Sample preparation and analysis

Analytical protocols used were as developed for marine oils during previous FRDC-funded projects (1991/77 and 1994/115) performed by CSIRO Marine Research. Details of all specific procedures are available in the FRDC final reports, the literature (e.g. Bakes and Nichols 1995; Bakes *et al.* 1995; Nichols *et al.* 1994;Volkman and Nichols 1991) and laboratory manuals. A description of the methods follows.

Oil was extracted from replicate specimens (up to n=3 for each species per sampling date) of individual species using the Bligh and Dyer (1959) one-phase methanol:chloroform:water extraction (2:1:0.8 v/v/v) procedure. 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 40°C. All samples were made up to a known volume in chloroform and stored at -20°C. TSE samples were stored for up to three days before oil analyses were commenced.

An aliquot of the TSE or total oil was analyzed using an Iatroscan MK V TH10 TLC-FID analyzer to determine the abundance of individual oil classes (Volkman and Nichols, 1991). Each replicate TSE sample of individual species was analysed in duplicate using silica gel SIII Chromarods (5 μm particle size). Samples were applied using 1 μL disposable micropipettes. Chromarods were developed in a glass tank lined with pre-extracted filter paper. The solvent system used for the oil separation was hexane-diethyl ether-acetic acid (60:17:0.2 v/v/v), a mobile phase resolving non-polar compounds such as wax esters (WE), triglycerides (TAG), free fatty acids (FFA) and sterols (ST) from polar lipid (PL). A second non-polar solvent system of hexane-diethyl ether (96:4 v/v) was also used for selected samples to resolve hydrocarbons (HC), WE and steryl esters (SE), and TAG and diacylglyceryl ethers (DAGE). 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 [e.g., phosphatidylcholine, cholesterol, cholesterol oleate, oleic acid, squalene, and TAG and DAGE (both purified from fish and deep-sea shark liver oil respectively); 0.1-10 μg range for each oil class]. The relative level of each oil class determined by TLC-FID represents the amount of each class divided by the sum of the individual oil classes. Peaks were quantified on a personal computer using DAPA software (Kalamunda, WA). Iatroscan results are generally reproducible to ±10% or better for individual lipid classes (Volkman and Nichols, 1991; Nichols *et al.* unpublished data).

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, FAME and free sterols 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. Samples were then treated with N,O-Bis(trimethylsilyl)-trifluoroacetamide (BSTFA) to form TMS derivatives (of free sterols) prior to instrumental analysis.

Gas chromatographic (GC) analyses of FAME and sterols were performed with a Hewlett Packard 5890A GC equipped with an HP-5 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 methyl tricosanoate

and/or methyl nonadecanoate 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 Millennium Scientific Software (Waters, USA). A previously characterized laboratory FAME standard was routinely run with sample batches to both assist with peak identification and assess GC performance, particularly the response for PUFA.

Verification of the identification of individual components was performed using GC-MS data and by comparing retention time data with those obtained for authentic and laboratory standards. GC results are subject to an error of ±5% for individual components. Gas chromatographic-mass spectrometric (GC-MS) analyses were performed on a Thermoquest GCQ GC-MS (Finnigan, USA) 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 column similar to that described above.

Average data (n=3) for each species is presented as percentage composition (e.g. of total fatty acids) and mg/100 g (wet weight) of flesh. Results are stored using the database packages, Excel and Quark. CSIRO may be approached for collaborative use of the database for further manipulation and presentation purposes.

6.3 Format of results on oil and fatty acid content and composition

The presentation of results in the literature on the oil and fatty acid content and composition of Australian seafoods varies (Nichols *et al.* 1998a). For example, whilst nearly all studies have reported oil content and percent fatty acid distribution, few studies have determined the absolute amount of individual or groups of fatty acids present in Australian fish. In some studies, absolute abundance of fatty acids was determined through estimation rather than by direct measurement. The uptake of dietary oil depends on the oil class distribution; in most studies to date on Australian seafood, oil class composition including cholesterol has not been reported.

In this study, results for individual species include oil content (percent of wet weight), oil composition including cholesterol (percent of total oil), fatty acid composition (percent of total fatty acids) and fatty acid content (mg/100 g wet weight). A representative data sheet for all parameters is shown in Table 7.2. Data for all species analysed is provided in *Seafood the Good Food II* (Mooney *et al.* 2002) prepared as part of this study.

The measurement and presentation of the wider range of oil parameters for seafood is now possible due to developments in methodology and instrumental procedures. The inclusion in this study of additional parameters is also due to the recognition by nutritionists of their importance to understand better the nutritional content and value of food items.

It was recently stated by Belling *et al.* (1997) that very little has been published on the fatty acid composition of Australian fish. In combination, the two volumes of *Seafood the Good Food* provides a comprehensive data set on the nutritional (oil) composition of principal Australian seafoods.

6.4 Fatty acid nomenclature

Fatty acids are designated by total number of carbons: number of double bonds, followed by the position of the first double bond (unsaturated centre) from the methyl (omega or n-) end of the molecule. For example, the structure of docosahexaenoic acid [22:6(n-3)] has 22 carbons, with 6 double bonds, the first double bond being 3 carbons from the methyl end of the molecule. The suffixes c and t indicate *cis* and *trans* geometry. The prefixes i, a and br indicate iso (2-methyl), anteiso (3-methyl) and branched respectively.

The term omega-3 fatty acid denotes PUFA with two or more *cis*-unsaturated centres, separated from each other by one methylene group and having the first unsaturated centre three carbons from the end methyl. Similarly, omega-6 denotes PUFA with two or more *cis*-unsaturated centres, separated from each other by one methylene group and having the first unsaturated centre six carbons from the end methyl. Further definitions and abbreviations are provided in Section 2.

6.5 Methods considerations

Tissue type

Large differences may occur in oil content and fatty acid composition depending on whether flesh only or flesh plus skin and subcutaneous fat are analysed (Naughton *et al.* 1983). As noted in Section 6.1, for fish analysed in this study, skin and subcutaneous fat were excluded from flesh samples. This decision was taken as it was assumed that the majority of consumers did not eat the skin.

Analytical procedures

Methods vary between studies for the extraction of oil from seafood, for the preparation of methyl esters and component identification. Similarly, the format for presentation of lipid and fatty acid compositional results on Australian species has varied (see Section 7.2).

In the preparation of methyl esters of fatty acids, problems with the quantitation of components (expressing data on a mg/100 g basis) have been observed and were recently raised at the Annual Meeting of the AAOCS. This issue also has been acknowledged by the AOCS and the current standard AOCS procedure is being evaluated (R. G. Ackman, personal communication). The methylation method used in this study was not the AOCS procedure, but was found to give reproducible and quantitative results in recent inter-laboratory comparative studies conducted in Australia and overseas (unpublished data).

Many early Australian studies were performed using packed columns which provide lower resolution than capillary columns used in this study. The higher resolution enables a greater number of minor components to be identified; results for these minor components may not be shown, but are included in total SAT, MUFA or PUFA (e.g. 12:0, i15:0, a15:0, i17:0, br19:0, 20:0, 22:0, 24:0, 14:1's, 18:1(n-5)c, 19:1's, 24:1, C_{16} PUFA). Values for total SAT, MUFA and PUFA therefore may be greater than the sum of the individual components shown. Several minor components were found to coelute under the GC conditions used, e.g.

a17:0 generally was not separated from 17:1(n-8)c; the former component was generally less abundant than 17:1 which is itself usually not >1%. For some samples, the C_{22} PUFA 22:5(n-6), when present as a minor component (approx. 0.1-1%), was found to coelute with DHA, particularly when the latter was a major constituent. Where this situation occurred, 22:5(n-6) was included with DHA.

Unusual fatty acids also have been identified in some species. However, their absence in most samples has resulted in these components not being included in the standard data sheets (Mooney *et al.* 2002). For example, non-methylene interrupted (NMI) diunsaturated C_{18}, C_{20} and C_{22} fatty acids were present in abalone and some other species. For these samples, the NMI acids are not shown in the data sheets as individual fatty acids, but they are included in the total level of PUFA and their presence has been footnoted.

The format of results used for this study includes a wider range of parameters than is usually reported. The inclusion of the additional parameters is largely due to the recognition by nutritionists of the importance of the determination of such parameters to better understand the nutritional content and value of food items.

Species identification and sample size

The importance of correct identification of fish has been previously noted by Brown *et al.* (1989) in a review of the fatty acid composition of Australian marine finfish. Scientific and marketing names and the Code for Australian Aquatic Biota (CAAB) used in fisheries data analysis are provided for each species analysed in this study together with oil compositional results (Nichols *et al.* 1998a; Mooney *et al.* 2002). This study was performed in parallel to the CSIRO Fish Handbook study (Yearsley *et al.* 1999) and genetic and other morphological techniques were used to confirm the identification of most species analysed.

Intra-species variation in lipid content and composition has been observed in many studies (e.g. Brown *et al.* 1989). In the present study, separate samples were obtained from individual specimens (generally n=3) and were separately analysed for each species. Analysis of a larger number of replicates was beyond the logistical and funding constraints of this study. Thus although standardised procedures have been adopted through this study, intra-species variation may still occur. Therefore the data reported herein serve as a guide for medical practicioners, nutritionists and other user groups and the fact that intra-species or other forms of variation may occur should be noted.

7. Oil composition of Australian seafood

Australians eat less seafood than they do beef, lamb or chicken. However, consumption has increased in the 1990s, partly due to the effective promotion of the health benefits of eating both finfishes and shellfishes. Nutritionists and medical authorities are encouraging people to eat seafoods because the oils contain high levels of nutritionally important omega-3 polyunsaturated fatty acids (PUFA).

In 1998 as part of FRDC project 1995/122, the content and composition of the oil from 200 species of Australian fishes, shellfishes and crustaceans were examined (Nichols *et al.* 1998a & b). In this study after consultation with industry, we have examined a further 79 species of seafood. The list of species analysed covers 58 fish, 9 cructaceans 9 shellfish and 3 other invertebrates (Table 7.1).

Collectively the two studies aim to:
1. determine the nutritional value of Australian species and how oil levels may differ with taxonomic group and other factors including geographical region;
2. transfer the knowledge to the Fishing Industry to better exploit the total catch, including waste products; and
3. provide oil compositional results to nutritionists and other consumer groups for use in communicating the health benefits of Australian seafood.

In this study, we have also examined:
4. the effects of aquaculture and processing (e.g., cooking, smoking, other forms of value-adding) and on the oil content and composition of Australian seafood. These findings are presented in Chapters 10–22.

7.1 Oils

In seafood, oils are the second largest component after protein. Oils have a variety of important roles; they serve as concentrated stores of energy, as fuel molecules and as components of cell membranes. Of main importance are triglycerides, polar oils and cholesterol.

Triglycerides serve as an energy store and polar oils and cholesterol are structural components of cell membranes. Cholesterol is a factor in coronary heart disease and other disorders when in dietary excess.

The main components of the oils are saturated and unsaturated fatty acids. EPA [eicosapentaenoic acid, 20:5(n-3)] and DHA [docosahexaenoic acid, 22:6(n-3)] are omega-3 polyunsaturated fatty acids (PUFA) and in the human diet are largely obtained from seafood. AA [arachidonic acid, 20:4(n-6)] is an omega-6 PUFA which is a precursor of prostaglandins (modulate hormone activity) and other eicosanoids (C20 physiologically active compounds). The human body manufactures only small amounts of these PUFA and we must therefore rely on dietary sources such as seafood.

The marine omega-3 PUFA have a wide range of potential health benefits, particularly with respect to the prevention of coronary heart disease and rheumatoid arthritis. They also may play a role against some forms of cancer and other disorders, although further research and

trials are required. Omega-3 PUFA also may be beneficial for infant brain and retina function and development.

7.2 Results and Discussion

Oil compositional profiles for all new species analysed in Project 1999/331 are presented in the companion Guide: *Seafood the Good Food II* (Nichols *et al.* 2002). A listing of species examined is shown in Table 7.1, with representative data (for grunter bream, *Pomadasys kaakan*) shown in Table 7.2.

A summary of the average content of omega-3 PUFA in various food groups is shown in Table 7.3. These results are for all seafood species reported in the two volumes of *Seafood the Good Food*, and indicate that seafood has considerably higher content of the beneficial omega-3 oils than other food groups. Wild-caught seafood containing an omega-3 PUFA content of greater than 300 mg/100 g (wet mass) are shown in Table 7.4.

It is noted that two species of escolar that rank in the top ten seafood species in omega-3 PUFA content have been associated with recent incidences of consumer illness. The illnesses have been attributed to the high wax ester content of escolar (see Chapters 8 and 9).

Summary findings for this study include:

- relative to other food groups, wild-caught, cultured and value-added seafood are the best and most readily available source of EPA and DHA;
- most Australian fish have high levels of omega-3 PUFA (average 235 mg/100 g, range 13 to 3760 mg/100 g) and low levels of cholesterol (average 28 mg/100 g);
- prawns have lower levels of omega-3 PUFA (average 130 mg/100 g) and higher levels of cholesterol (average 130 mg/100 g) than fish;
- Australian fish generally have higher relative levels of DHA than fishes from the Northern Hemisphere;
- fish from warmer waters generally have lower omega-3 / omega-6 ratios than fish from temperate waters, due largely to higher relative levels of AA;
- fish generally contain polar oil and/or triglyceride, although a few species contain unusually high content of wax ester, hydrocarbon or diacylglyceryl ether
- cultured (farmed) seafood has excellent potential to be an excellent source of omega-3 PUFA, as the oil in the feed can be manipulated to increase oil levels in products;
- cooking and processing have no discernable effect on the content and composition of the omega-3 PUFA in seafood; and
- Some variation was observed with season and location for selected fish and shellfish, but the differences generally had little effect on oil quality.

Further details on the results of this study and an earlier study are available in Nichols *et al.* (1998b) *Seafood the Good Food*; Nichols *et al.* (1998a) FRDC Final Report 95/122; Yearsley *et al.*(1999) *Australian Seafood Handbook*; Mooney *et al.* (2002) *Seafood the Good Food II.*

Table 7.1. Species analysed during project 1999/331.

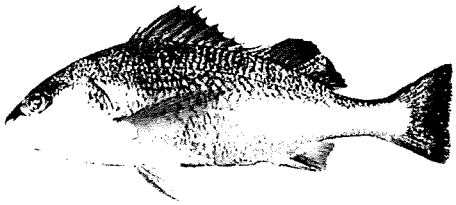
Marketing name	*Scientific name*	CAAB
CARTILAGINOUS FISHES		
spikey dogfish	*Squalus megalops*	37 020006
skate	*Raja* sp.	37 031005
elephant fish	*Callorhinchus milii*	37 043001
BONY FISHES		
longfin eel	*Anguilla reinhardtii*	37 056002
whitebait	*Galaxias maculatus*	37 102006
whitebait	*Lovettia sealii*	37 103002
freshwater catfish	*Tandanus tandanus*	37 192006
ribaldo	*Mora moro*	37 224002
southern rock cod	*Pseudophycis barbata*	37 224003
garfish	*Arrhamphus sclerolepis*	37 234006
alfonsino	*Beryx splendens*	37 258002
moonfish	*Lampris guttatus*	37 268001
Australian bass	*Macquaria novemaculeata*	37 311034
barramundi cod	*Cromileptes altivelis*	37 311044
coral cod	*Cephalopholis sonnerati*	37 311045
Murray cod	*Maccullochella peelii*	37 311076
Maori rockcod	*Epinephelus undulatostriatus*	37 311086
Striped perch	*Scortum barcoo*	37 321025
redfin	*Perca fluviatilis*	37 329001
school whiting	*Sillago bassensis*	37 330002
black kingfish	*Rachycentron canadum*	37 335001
samson fish	*Seriola hippos*	37 337007
samson fish	*Seriola dumerili*	37 337025
queenfish	*Scomberoides commersonnianus*	37 337032
turrum	*Carangoides fulvoguttatus*	37 337037
bigeye trevally	*Caranx sexfasciatus*	37 337039
dart	*Trachinotus botla*	37 337066
green jobfish	*Aprion virescens*	37 346027
king snapper	*Pristipomoides filamentosus*	37 346032
ruby snapper	*Etelis coruscans*	37 346038
sweetlip bream	*Diagramma labiosum*	37 350003
grunter bream	*Pomadasys kaakan*	37 350011
mulloway	*Argyrosomus hololepidotus*	37 354001
black jewfish	*Protonibea diacanthus*	37 354003
boarfish	*Pentaceropsis recurvirostris*	37 367003
bigspine boarfish	*Pentaceros decacanthus*	37 367004
banded morwong	*Cheilodactylus spectablis*	37 377006
red morwong	*Cheilodactylus fuscus*	37 377009
trumpeter	*Latridopsis forsteri*	37 378002
king threadfin	*Polydactylus sheridani*	37 383005
wrasse	*Notolabrus tetricus*	37 384003
wrasse	*Notolabrus gymnogenis*	37 384041

Table 7.1 (continued)

Maori wrasse	*Oxycheilinus digrammus*	37 384065
stargazer	*Kathetostoma canaster*	37 400018
rabbitfish	*Siganus* sp.	37 438902
escolar	*Ruvettus pretiosus*	37 439003
escolar	*Lepidocybium flavobrunneum*	37 439008
ribbonfish	*Lepidopus caudatus*	37 440002
skipjack tuna	*Katsuwonus pelamis*	37 441003
slender tuna	*Allothunnus fallai*	37 441021
rudderfish	*Tubbia tasmanica*	37 445002
rudderfish	*Centrolophus niger*	37 445004
rudderfish	*Tubbia* sp.	37 445903
velvet leatherjacket	*Parika scaber*	37 465005
leatherjacket	*Pseudomonacanthus peroni*	37 465020
leatherjacket	*Aluterus monoceros*	37 465022
reef leatherjacket	*Meuschenia freycineti*	37 465036
CRUSTACEANS		
royal red prawn	*Haliporoides sibogae*	00 701004
freshwater prawn	*Macrobrachium rosenbergii*	00 701113
school prawn	*Metapenaeus macleayi*	00 701321
sand crab	*Ovalipes australiensis*	00 702905
scampi	*Metanephrops velutinus*	00 703005
scampi	*Metanephrops boschmai*	00 703006
eastern rock lobster	*Jasus verreauxi*	00 703013
Balmain bug	*Ibacus peronii*	00 703028
marron	*Cherax tenuimanus*	00 704004
MOLLUSCS		
cuttlefish	*Sepia pharaonis*	00 610001
cuttlefish	*Sepia rex*	00 610007
northern calamari	*Sepioteuthis lessoniana*	00 620011
saucer scallop	*Amusium balloti*	00 651002
saucer scallop	*Amusium pleuronectes*	00 651003
cockle	*Katelysia scalarina*	00 657004
Roe's abalone	*Haliotis roei*	00 662003
brownlip abalone	*Haliotis conicopora*	00 662004
OTHER INVERTEBRATES		
jellyfish	*Aurelia aurita*	00 670002
beche-de-mer	*Holothuria scabra*	00 708103
sea urchin	*Heliocidaris erythrogramma*	00 711002
IMPORTED SPECIES		
Nile perch	*Lates niloticus*	
greenlip mussel	*Perna canaliculus*	

Table 7.2. Representative oil content and composition profile (for Grunter bream). Profiles for all species analysed in Project 1999/331 are provided in *Seafood the Good Food II* (Mooney *et al.* 2002).

Grunter bream *Pomadasys kaakan* 37 350011



Summary

Oil	**0.6 %**
	mg/100g (wet)
20:4(n-6) AA	26
20:5(n-3) EPA	9
22:6(n-3) DHA	44
(n-3)/(n-6)	1.5

Oil Content

Oil Class	mg/100g (wet)
Total oil	**588**
Wax ester	0
Triglyceride	2
Free fatty acid	10
Cholesterol	33
Polar oil	543
Fatty Acids	
Total fatty acids	**206**
Total saturates	67
Total monounsaturates	42
Total polyunsaturates	97
Total (n-3)	58
Total (n-6)	38

Fatty Acid Composition

Fatty Acid	Percent (%)
Saturates	
14:0	1.5
15:0	0.3
16:0	17.4
17:0	0.7
18:0	11.9
20:0	0.1
22:0	0.2
24:0	0.2
Total	**32.6**
Monounsaturates	
16:1(n-5)	0.1
16:1(n-7)	1.6
16:1(n-9)	0.1
17:1(n-8)	0.5
18:1(n-7)	2.0
18:1(n-9)	11.0
20:1(n-7)	0.1
20:1(n-9+n-11)	1.0
22:1	1.5
24:1	2.5
Total	**20.4**
Polyunsaturates	
18:2(n-6)	0.3
18:3(n-3)	0.3
18:3(n-6)	0.1
18:4(n-3)	0.1
20:2(n-6)	0.3
20:3(n-6)	0.2
20:4(n-3)	0.2
20:4(n-6) AA	12.7
20:5(n-3) EPA	4.2
22:3(n-3)	0.0
22:4(n-6)	2.1
22:5(n-3)	2.2
22:5(n-6)	2.9
22:6(n-3) DHA	21.3
Total	**47.0**

2

Table 7.3. Summary of average content of omega-3 PUFA in Australian seafood and comparison to other food groups.

Food group	omega-3 PUFA mg/100 g (wet weight)
Fish	**235**
Oysters	**150**
Prawns	**130**
Lobster	**105**
Turkey	35
Beef	22
Chicken	19
Lamb	18
Pork	0
Veal	0

Data for non-seafood items from references cited in Nichols *et al.* (1998a)

Table 7.4. Wild-caught seafood containing omega-3 PUFA content of greater than 300 mg/100 g (wet mass)

Marketing name	Scientific name	Oil (%)	Total (n-3)
slender tuna	*Allothunnus fallai*	16.5	3759
swordfish	*Xiphias gladius*	7.7	1021
escolar*	*Ruvettus pretiosus*	17.8	1019
banded morwong	*Cheilodactylus spectablis*	3.2	820
alfonsino	*Beryx splendens*	5.2	796
whitebait	*Lovettia sealii*	2.6	734
escolar*	*Lepidocybium flavobrunneum*	19.2	717
big-eye trevally	*Caranx sexfasciatus*	4.7	709
whitebait	*Galaxias maculatus*	3.3	687
blue mackerel	*Scomber australasicus*	3.8	508
Australian bonito	*Sarda australis*	1.5	433
gemfish	*Rexea solandri*	2.6	424
rudderfish	*Centrolophus niger*	14.4	415
Spanish mackerel	*Scomberomorus commerson*	3.0	383
sweep	*Scorpis lineolatus*	1.3	370
Australian herring	*Arripis georgianus*	1.7	359
Western blue grouper	*Achoerodus gouldii*	3.6	358
bigspine boarfish	*Pentaceros decacanthus*	1.5	354
Eastern Australian salmon	*Arripis trutta*	1.1	338
spotted mackerel	*Scomberomorus munroi*	1.2	335
school mackerel	*Scomberomorus queenslandicus*	1.1	328
grey mackerel	*Scomberomorus semifasciatus*	1.1	328
tailor	*Pomatomus saltatrix*	1.3	326
threadfin emperor	*Lethrinus genivittatus*	2.6	325
bight redfish	*Centroberyx gerrardi*	0.5	322
pilchard	*Sardinops neopilchardus*	1.2	312
blue eye trevalla	*Hyperoglyphe antarctica*	1.3	314

* consumption of escolar may cause illness (Yearsley *et al.* 1999)

8. Unusually high levels of non-saponifiable lipids in the fishes escolar and rudderfish: Identification by gas and thin layer chromatography

Chapter 8 published as:
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8.1 Summary

Analysis of the non-saponifiable lipids of the fishes *Lepidocybium flavobrunneum* and *Ruvettus pretiosus* (escolar), and *Centrolophus niger* and *Tubbia* spp (rudderfish) was performed. The analyses were used to clarify the cause of recent reports of illness (diarrhoea) in Australia from consumption of purported rudderfish. Both escolar and rudderfish contained very high levels of oil (generally between 14 to 25%, as % wet weight) in the fillet and the oil compositions were different to most seafood. Escolar oil contained mainly wax ester (>90% of oil). The oil from five specimens of rudderfish contained mainly diacylglyceryl ether (DAGE, >80% of oil) or hydrocarbon (> 80% of oil, predominately squalene). One rudderfish specimen contained mainly polar lipid. Major differences in oil content and composition, including fatty alcohol and glyceryl ether diols (derived from DAGE), were observed between purported individuals of the same species or related species of rudderfish, raising the possibility of geographic or seasonal differences affecting the oil composition. The oil composition of fish fillet samples associated with the health issues was consistent with the profiles for escolar, rather than rudderfish species. These findings, in particular the lipid class and fatty alcohol profiles, were supported by general protein fingerprinting results and were consistent with the samples originating from individuals of the escolar species *Lepidocybium flavobrunneum*. The high wax ester content of the escolar group clarifies the reported diarrhoeal effects to consumers. Purgative properties of high wax ester containing fish oils have been reported for escolar and other species. The results highlight the potential for non-saponifiable lipid profiles to be used for identification of fish fillets and oils to at least group level.

Keywords: fish lipids, squalene, wax ester, diacylglyceryl ether, *Lepidocybium flavobrunneum*, *Ruvettus pretiosus*, *Centrolophus niger*, *Tubbia* spp

8.2 Introduction

Marine seafoods are increasingly marketed for their health benefits to consumers (eg. Kinsella 1987, Howe 1998). However, consumption of a very limited number of species has also been reported to produce purgative properties in some consumers, with evidence including results of rat-feeding studies, e.g. for the escolar *Lepidocybium flavobrunneum* and *Ruvettus pretiosus* (Ochiai *et al.* 1983, Kinumaki *et al.* 1977). In general this effect is reported to be due to the presence of wax ester-rich oils in the flesh (Cox *et al.* 1932).

Health complaints have occurred recently in Australia associated with the consumption of fillets sold as rudderfish. The marketing group rudderfish consists of species from three trevalla (family *Centrolophidae*) genera, *Centrolophus*, *Schedophilus* and *Tubbia*, with several undescribed species and uncertain distribution in Australian waters. Consumers had purchased "rudderfish" fillets and, after cooking and eating, some had suffered severe diarrhoeal effects sometimes associated with the fish (Yearsley *et al.* 1999). However, concern existed over the species identity and the cause of the health effects, particularly as both groups are caught as long-line by-catch and anedoctal evidence suggested that escolar species were being sold as rudderfish. The name "escolar" in Australian fisheries includes two known gemfish (family *Gempylidae*) species, *Lepidocybium flavobrunneum* and *Ruvettus pretiosus*. The latter species has also been referred to as "castor oil fish (Nevenzal *et al.* 1965). Both species have reported purgative properties (Berman et. al 1981).

The lipid composition of *R. pretiosus* obtained from a Tokyo, Japan fish market has been reported (Nevenzal *et al.* 1965). However, prior to this study, the oil content and composition of Australian escolar and rudderfish were not available. We present here a comparison of the oil content and composition profiles, with particular emphasis on the non-saponifiable lipids, of two fish fillets associated with consumer illness, and those obtained for reference samples from the escolar and rudderfish groups. The analyses, supported by general protein fingerprinting (unpublished data) of the samples, highlight the potential for non-saponifiable lipid profiles to be used for identification of selected escolar and rudderfish samples to at least group level.

8.3 Experimental

Samples

Two frozen fillet samples of unknown species were air freighted to Hobart, Tasmania from the South Australian Public and Environmental Health Service – unknown samples 1 and 2. Both samples were associated with cases of severe diarrhoea in consumers. Reference samples (2-3 specimens of each species, Table 8.1) of muscle tissue of escolar species *Ruvettus pretiosus* (Cocco, 1829) and *Lepidocybium flavobrunneum* (Smith 1849), and rudderfish species *Centrolophus niger* (Gmelin, 1789) and *Tubbia* spp. had been stored at –80°C at the CSIRO Marine Laboratories (Yearsley *et al.* 1999) and were used for comparison of oil content and composition. Skin, including lipid deposits, was removed from the samples analysed in this study.

Lipid extraction and fractionation

Small samples of flesh without skin were quantitatively extracted using a modified Bligh and Dyer (Bligh *et al.* 1959) one-phase methanol/chloroform/water extraction (2:1:0.8, by vol); the 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, by vol,

methanol/chloroform/water). The total lipid was concentrated (i.e. solvents removed *in vacuo*) using rotary evaporation at 40°C. Lipid class analyses were conducted within three days, with samples stored in a known volume of chloroform.

An aliquot of the total lipid was analyzed using an Iatroscan MK V TH10 thin-layer chromatography–flame-ionization detector (TLC–FID) analyzer (Tokyo, Japan) to determine the abundance of individual lipid classes (Volkman *et al.* 1991). Samples were applied in duplicate or triplicate to silica gel SIII Chromarods (5 μm particle size) using 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, by vol), a mobile phase resolving non-polar compounds such as wax esters (WE), triacylglycerols (TAG), free fatty acids (FFA) and sterols (ST). A second non-polar solvent system of hexane/diethyl ether (96:4 vol/vol) was also used to separate hydrocarbon (HC) from WE and TAG from diacylglyceryl ether (DAGE). After development, the chromarods were oven-dried and analyzed immediately to minimize adsorption of atmospheric contaminants. The FID was calibrated for each compound class [phosphatidylcholine, cholesterol, cholesteryl ester, oleic acid, squalene, wax ester (derived from fish oil), triacyclglycerol (derived from fish oil) and DAGE (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 *et al.* 1991).

An aliquot of the total lipid was saponified in an aqueous solution of methanolic KOH (80/20/5, MeOH/H_2O/KOH, v/v/w; 60°C, 3 h). The non-saponifiable lipids were extracted with hexane/chloroform (4:1, v/v, 3x) and treated with N,O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA, 60°C, 12 h) to convert alcohols, sterols and glyceryl ether diols to their corresponding O-TMSi (trimethylsilyl) ethers. With the wax ester rich species, the saponification step was not used, as the reaction was incomplete. Rather, the lipid was treated with methanol/hydrochloric acid/chloroform (10:1:1, by vol; 80°C, 2 h). The fatty acid methyl esters (FAME) and alcohol products were extracted into hexane/chloroform (4:1, v/v, 3x) and the mixture treated with BSTFA as above to convert alcohols and sterols to their corresponding O-TMSi (trimethylsilyl) ethers.

Gas chromatography and gas chromatography – mass spectrometry

Gas chromatographic (GC) analyses of non-saponifiable lipids were performed with a Hewlett Packard 5890A GC (Avondale, PA) equipped with an HP-5 cross-linked methyl silicone fused silica capillary column (50 m × 0.32 mm i.d.), an FID, a split/splitless injector and an HP 7673A auto sampler. Hydrogen was the carrier gas. Following addition of methyl nonadecanoate and methyl tricosanoate internal standards, 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^{-1}, then to 250°C at 2°C min^{-1} and finally to 300°C at 5°C min^{-1}. Peaks were quantified with Waters Millenium software (Milford, MA, USA). Individual components were identified using mass spectral data and by comparing retention time data with those obtained for authentic and laboratory standards. GC results are subject to an error of ±5% of individual component abundance. GC–mass spectrometric (GC–MS) analyses were performed on a Finnigan Thermoquest GCQ GC-mass spectrometer (Austin, TX, USA) fitted with an on-column injector. The GC was fitted with a capillary column similar to that described above.

8.4 Results and Discussion

Escolar

Oil content of reference samples of the two escolar species (*L. flavobrunneum* and *R. pretiosus*) was in the range 18% to 21% (as % wet weight, Table 8.1). These values represent to our knowledge one of the highest oil content values recorded for wild-caught Australian fishes (200 species, mean 1%, range 0.3% to 8%) (Nichols *et al.* 1998, Nichols *et al.* 1998). Wax ester was the dominant oil class in all escolar samples (90 to 97% of oil, Table 8.1, Figure 8.1).

With the wax ester rich species, a saponification step was not used to obtain the non-saponifiable lipids, as we observed that the reaction was incomplete. Instead we used a direct transmethylation procedure. This procedure provides a mixture of FAME and fatty alcohols (Figure 8.2). The main fatty alcohols, in decreasing order of abundance, in *L. flavobrunneum* were 16:0 (53%, mean value), 18:1(n-9) (21%), 18:0 (9%), 18:1(n-7) (3.3%), 16:1(n-7) (3.1%), 14:0 (2.6%) and 20:1 (n-9) (2.5%) (Table 2). These seven components accounted for approximately 94% of the total fatty alcohols present. The profile for *R. pretiosus* was very similar, although relatively higher levels of 18:1(n-9) and 16:1(n-9), and lower levels of 18:0 were observed. A similar fatty alcohol profile was also reported for muscle wax ester of *R. pretiosus* obtained from a Japanese fish market (Nevenzal *et al.* 1965).

Several reports document the fatty alcohol profiles of Japanese specimens of *L. flavobrunneum* (e.g. Mori *et al.* 1966, Kawai *et al.* 1985). These earlier studies did not distinguish the different isomers of the C_{16}, C_{18}, C_{20} and C_{22} monounsaturated fatty alcohols. As noted above, we have used several of these specific components to distinguish the two escolar, *L. flavobrunneum* and *R. pretiosus.* One Japanese profile showed markedly higher levels of 20:1 and 22:1 (16%), with lower levels of 16:0 (34%) (Mori *et al.* 1966) than our findings. The other profile showed higher 16:0 (65%) and lower 18:1 (16%), with similar levels of 20:1 and 22:1 to our findings (Kawai 1985). The reason(s) for these differences may be due to environmental factors such as catch location and/or diet of the fish, and/or analytical factors. It remains to be determined whether and how such differences in fatty alcohol profiles may influence the purgative properties of these species.

The wax ester-derived fatty alcohol profiles of *L. flavobrunneum* and *R. pretiosus* are also readily distiguished from those of other wax ester-rich fish. For example, orange roughy (*Hoplostethus atlanticus)* and six species of deep-sea oreos collected from Australian waters contained considerably higher levels of C_{20} and C_{22} monounsaturated fatty alcohols (40 to 90%) (Bakes *et al.* 1995, Elliott *et al.* 1990) compared to the escolar species (2 to 6%, Table 8.2).

Rudderfish

In contrast to the lipid class profile for escolar, wax ester was either absent or only a minor component in the lipids of the rudderfish *C. niger* and *Tubbia* spp. (Table 8.1, Figure 8.1). With the exception of one specimen of *C. niger,* oil content in both species was in the range 14% to 25% (% wet weight). Once again, the oil content of these fishes is considerably higher than previously reported for Australian fishes. For *C. niger*, the two oil-rich specimens contained high levels of diacylglyceryl ether (DAGE, 93%, as % of total oil).

The third specimen of *C. niger* was lower in oil content (1.7%) and contained only 17% DAGE, with 42% polar lipid (PL) and 22% free fatty acid (FFA) (Table 8.1).

Glyceryl ether diols (GED) are formed by saponification of DAGE. GED profiles obtained for the DAGE-containing rudderfish are provided in Table 8.3, with a representative GC trace shown in Figure 8.3. The GED profiles for the rudderfish *C. niger* are generally simpler than the fatty acid (data not shown) and fatty alcohol profiles. The main GED, in decreasing order of abundance, were 18:1(n-9), 16:0, 18:0, 18:1(n-7) and 16:1 (Table 8.3). Considerable variation was observed between the three *C. niger* samples in the relative levels of these and other minor GED. This finding will be discussed further below.

Tubbia specimens differed from both escolar and *C. niger*. Hydrocarbon (HC) was the main oil class (mean 87%, as % total oil) present in two specimens. The predominant component in the non-saponifiable lipids was identified by GC analysis as squalene (Figure 8.4). A third specimen did not contain hydrocarbon. Rather this sample was rich in DAGE (82%).

When a transmethylation procedure was used on the total lipid of the two HC-rich samples, we observed virtually complete degradation of squalene. We are aware that squalene levels in certain marine oils may sometimes be measured after the use of a transmethylation procedure (e.g. BF_3/methanol; Glen Neill, personal communication); our findings indicate that care is needed should such procedures be used.

The high levels of squalene in the flesh of *Tubbia* is extremely unusual. Although squalene is common in liver oils of deep sea sharks (Bakes *et al.* 1995), where it is metabolically inert and its only known function is in bouyancy (Phleger 1991, Phleger 1998), its presence as the predominant lipid in *Tubbia* spp. muscle is unique. One other study has reported squalene at 12% of the lipid in the head and body of the teleost eulacon *Thaleichthys pacificus* (Ackman *et al.* 1968); the main lipid class was triglyceride (84%). The role of squalene in *T. pacificus* was suggested to be to dilute and reduce the viscosity or melting point of the triglyceride in the marine life phase in cold ocean water. It may be speculated that the unusually high levels of squalene in *Tubbia* may have a similar bouyancy or other physiological role, however, at this stage the precise role remains to be determined.

The GED profile of *Tubbia* spp. was generally similar to *C. niger*, although the relative level of individual components varied between individuals. The main GED, in decreasing order of abundance, were 16:0, 18:1(n-9), 18:0, 18:1(n-7) and 16:1 (Table 8.3). The individual variation may be a reflection of species or geographic differences; additional taxonomic and lipid compositional research is required for the rudderfish.

Given the differences observed between individual rudderfish specimens of the same species (*Tubbia* sp. and *C. niger)*, a larger number of authentic samples will need to be be examined to fully chemotaxonomically characterize these species.

Unknown samples

Both of the unknown fillet samples that had been associated with consumer illness had a very high oil content (>22%, as % wet weight) and the dominant lipid class was wax ester (97%, as % of total oil, Table 8.1). Such a high oil content and unusual composition were similar to that found in the reference collection samples for both escolar species (Table 8.1).

Although the lipid class and fatty alcohols profiles for *L. flavobrunneum* and *R. pretiosus* are very similar, higher levels of 18:1(n-9) and 16:1(n-9), and lower levels of 18:0 were observed in *R. pretiosus* as noted above. Based on comparison of the fatty alcohol profiles, the unknown samples grouped more closely with *L. flavobrunneum* than with *R. pretiosus* (Table 8.2). This finding is consistent with results obtained from general protein fingerprinting of the two unknown (unpublished data) and the reference samples (Yearsley 1999).

Purgative and other properties of the oils

The first report on the purgative properties of *R. pretiosus* occurred in 1841 (Lowe 1841). More recently, and based on the high wax ester content in Japanese specimens of *L. flavobrunneum* and *R. pretiosus* (Nevenzal *et al.* 1965), the diarrhoea and seborrhoea-producing activity of these fishes was investigated (Mori *et al.* 1966). Based on the results for feeding trials in rats, the flesh and acetone-derived oil of both species were deemed not suitable for human food. However, the flesh of sun-dried fish is believed to be less harmful, as oil easily leaks out during sun drying (Mori *et al.* 1966).

The high levels of wax ester rich oil in orange roughy has been previously reported (fillets 7-10% oil; oil composition 95% wax ester) (Bakes *et al.* 1995). Extrapolating results obtained from feeding growing rats and pigs with orange roughy, it was proposed that "normal consumption" of orange roughy by humans was unlikely to cause serious health problems (James *et al.* 1986). However, the level of wax ester oil in escolar (18-24%) is nearly three times greater than in orange roughy.

Wax ester oils derived from orange roughy and oreo dories have been incorporated into several industrial cleaning, degreasing and other products in the past decade (Elliott *et al.* 1990, Nichols *et al.* 1997). Based on the findings of this study, escolar may represent an additional source of wax ester oils for consideration by industry.

Capsules of DAGE and hydrocarbon oils derived from the livers of deep-sea sharks are marketed as neutraceuticals for human consumption. Based on the findings of our study, rudderfish represent an additional source of the DAGE oils. The health benefits proposed to be associated with shark liver oils include an enhanced immune system, antibacterial activity, and possible regression of tumour growth and radiation induced damage (e.g. Brohult 1962, Brohult *et al.* 1986). Purgative effects of DAGE and hydrocarbon (squalene) oils have not to our knowledge been reported, however, the effect of intake of such oils at levels of 14% or greater are unknown. Therapeutic doses of these oils are of the order of 1 to 5 g per day, whilst 14% of a 150 g serving would be considerably greater (approximately 20 g).

8.5 Conclusions

Our study provides a bench mark for the use of lipid profiles, in particular the non-saponifiable lipids, for the identification of fish fillets and oils to at least group level. Members of the escolar and rudderfish groups are unusual in containing very high levels of oil (14-25%, as % of total oil) and having oil composition profiles different to most seafood. Oils from seafood, with some exceptions, are generally rich in triacylglycerol and / or phospholipid. The unusual oil profiles of specific members of the escolar and rudderfish groups are not consistent within their families. Members of both families (*Gempylidae* and *Centrolopidae*) are known to have more conventional triacylglycerol or phospholipid oils (Nichols *et al.* Nichols *et al.* 1998). Oil from

escolar species was rich in wax ester, for which purgative properties have been previously reported. Rudderfish species in contrast contained mainly diacylglyceryl ether (DAGE) or hydrocarbon (predominantly squalene). Purgative effects of DAGE and hydrocarbon oils have not to our knowledge been reported and capsules of these oils derived from the livers of deep-sea sharks are marketed as nutraceuticals for human consumption. The high wax ester oil composition results obtained for two unknown fillet samples clarify the reported diarrhoeal effects on consumers. The results and possible incorrect naming of the fillets suggest that consumers should be made aware of the oil type in these two groups and that strict use be made of recommended marketing names to avoid similar health issues. Oil composition results support genetic and taxonomic evidence (unpublished) that several undescribed species may exist in the rudderfish and escolar groups. Further specific taxonomic research on these groups is required, together with comparison of the oil profiles between and within (geographic and seasonal) species. In addition, insight into the physiological basis for the occurrence of these very different oils in the muscle of teleosts would be valuable.

8.6 Acknowledgement

This work was supported by the Australian Fisheries Research and Development Corporation - project 99/331. Ross Daley and other CSIRO Marine Research colleagues provided invaluable input. Drs Rick Phleger and Patti Virtue provided valuable comments on the manuscript, as did two anonymous referees and the Symposium Volumes Editor, Dr Erich Heftmann. Danny Holdsworth managed the CSIRO Marine GC-MS facility. The South Australian Public and Environmental Health Service provided the unknown fish samples.

Table 8.1. Oil class composition and content of escolar and rudderfish (oil class determined by TLC-FID)

Sample	Oil class composition (% of oil)							*Total oil*[#] *(% wet weight)*
	HC	WE	TAG	DAGE	FFA	ST	PL	
Unknown (1)*	-	98.1	-	-	0.4	0.2	1.3	22.1
Unknown (2)	-	97.5	-	-	0.3	0.2	2.0	24.5
Escolar								
L. flavobrunneum(1)	-	96.1	-	-	0.7	0.4	2.8	18.9
L. flavobrunneum(2)	-	96.6	-	-	-	0.2	3.2	20.3
R. pretiosus (1)	-	96.9	-	-	0.6	0.4	2.1	21.2
R. pretiosus (2)	1.1	90.1	1.5	0.5	0.7	0.6	5.7	ND
R. pretiosus (3)	-	95.8	0.4	-	0.4	0.5	2.9	17.8
Rudderfish								
C. niger (1)	0.7	-	4.5	92.5	0.6	0.3	1.4	14.1
C. niger (2)	-	1.5	13.0	17.4	21.6	4.3	42.2	1.7
C. niger (3)	-	-	9.7	87.5	1.0	0.5	1.3	19.8
Tubbia *sp.* (3)	80.5	-	10.5	6.0	0.6	0.3	2.1	20.5
Tubbia *sp.* (4)	93.4	-	0.3	2.2	0.7	0.2	3.2	15.9
Tubbia *sp.* (8)		-	-14.9	82.1	0.9	0.4	1.7	24.8

Abbreviations: HC, hydrocarbon; WE, wax ester; TAG, triglyceride; DAGE, diacylglyceryl ether; FFA, free fatty acid; ST, sterol; PL, polar lipid; ND, not determined; - , not detected.

* Denotes sample reference number.

Gravimetric determination.

Table 8.2. Fatty alcohol composition of escolar and rudderfish (derived from wax esters and determined by GC analysis)

Fatty alcohol	Percentage composition							
	Escolar *Lepidocybium flavobrunneum*		Unknown		Escolar *Ruvettus pretiosus*			Rudderfish *Centrolophus niger*
	(1)*	(2)	(1)	(2)	(1)	(2)	(3)	(2)
14:0	2.9	2.4	3.6	3.7	2.6	3.4	2.8	0.0
a15:0	0.1	0.1	0.1	0.3	0.1	0.2	0.2	0.0
15:0	1.0	0.9	1.0	1.3	0.9	1.1	1.2	0.0
i16:0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0
16:1(n-9)	0.4	0.2	0.4	0.3	1.1	0.4	0.9	0.0
16:1(n-7)	3.4	2.8	3.8	2.8	3.5	4.4	3.5	2.7
16:1(n-5)	0.6	0.3	0.5	0.3	0.3	0.6	0.6	0.0
16:0	53.2	51.9	47.9	44.4	48.0	48.5	46.8	41.9
i17:0	0.4	0.4	0.3	0.7	0.6	0.4	0.8	0.0
a17:0	1.1	1.0	1.2	1.2	1.2	1.1	1.3	0.0
17:0	1.0	1.1	1.1	1.4	1.1	1.0	1.2	0.0
i18:0	0.2	0.5	0.4	0.5	0.5	0.3	0.4	0.0
18:1(n-9)	21.4	21.1	22.9	20.1	26.4	26.6	25.8	28.6
18:1(n-7)	3.4	3.2	3.4	3.3	3.7	3.7	3.5	21.6
18:1(n-5)	0.3	0.4	0.2	0.2	0.3	0.2	0.3	0.0
18:0	8.3	9.4	8.5	10.4	6.4	6.1	7.7	5.2
20:2	0.3	0.4	0.4	0.4	0.4	0.1	0.4	0.0
20:1(n-9)	1.7	3.3	3.8	8.0	2.5	1.5	1.6	0.0
20:1(n-7)	0.1	0.2	0.2	0.3	0.1	0.1	0.2	0.0
20:0	0.2	0.4	0.3	0.4	0.3	0.3	0.2	0.0
22:1	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

*Denotes sample reference number.

Table 8.3. Glyceryl ether diol composition (derived from DAGE) of escolar and rudderfish (determined by GC analysis)

Glyceryl ether diol	Percentage composition						
	Escolar *Ruvettus pretiosus*	Rudderfish *Centrolophus niger*			Rudderfish *Tubbia spp.*		
	(3)*	(1)	(3)	(2)	(3)	(8)	(4)
14:0	0.0	1.5	1.4	3.2	3.9	0.0	1.7
15:0	0.0	0.7	0.8	1.8	2.0	0.0	1.9
16:1	4.2	2.8	2.5	5.0	3.2	3.2	2.8
16:0	45.7	14.9	15.9	25.1	37.6	32.1	32.3
17:0	0.0	1.2	1.1	1.6	1.9	0.0	1.5
17:0	0.0	0.8	0.8	1.5	1.1	0.0	1.2
18:1(n-9)	34.0	61.7	61.2	41.3	21.6	29.5	40.1
18:1(n-7)	4.2	3.8	3.7	5.4	2.9	3.3	4.8
18:0	11.9	11.4	11.5	15.2	25.8	31.9	12.9
20:1	0.0	1.2	1.1	0.0	0.0	0.0	0.8
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0

* Denotes sample reference number.

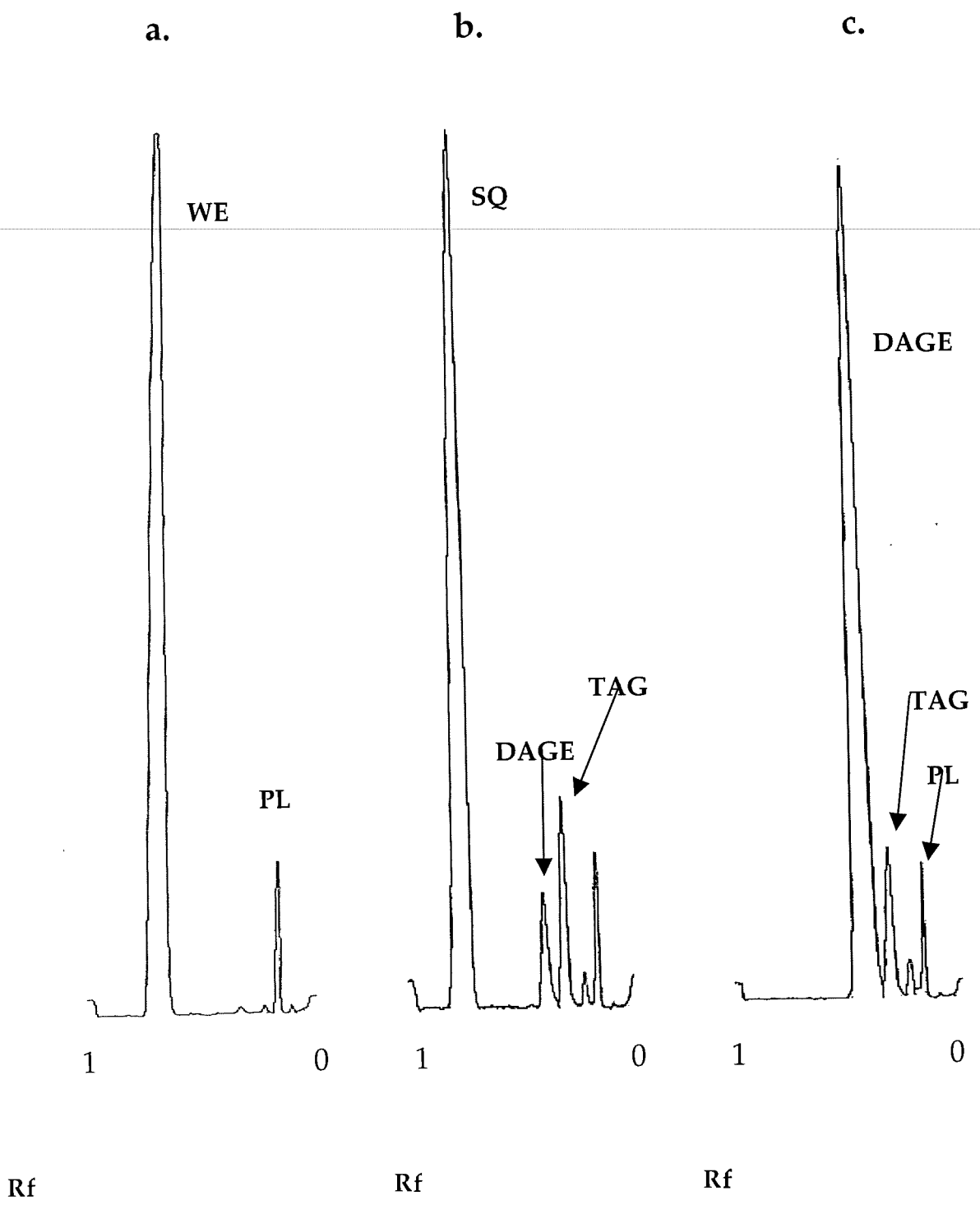


Figure 8.1. TLC-FID chromatograms showing lipid class distributions for escolar: a. *Lepidocybium flavobrunnen*, and rudderfish: b. *Tubbia* spp. and c. *Centrolophus niger*. Abbreviations: WE, wax ester; PL, phospholipid; SQ, squalene; DAGE, diacylglyceryl ether; TAG, triacylglycerol; Rf, retention factor. Solvent: hexane/diethyl ether, 96/4, v/v.

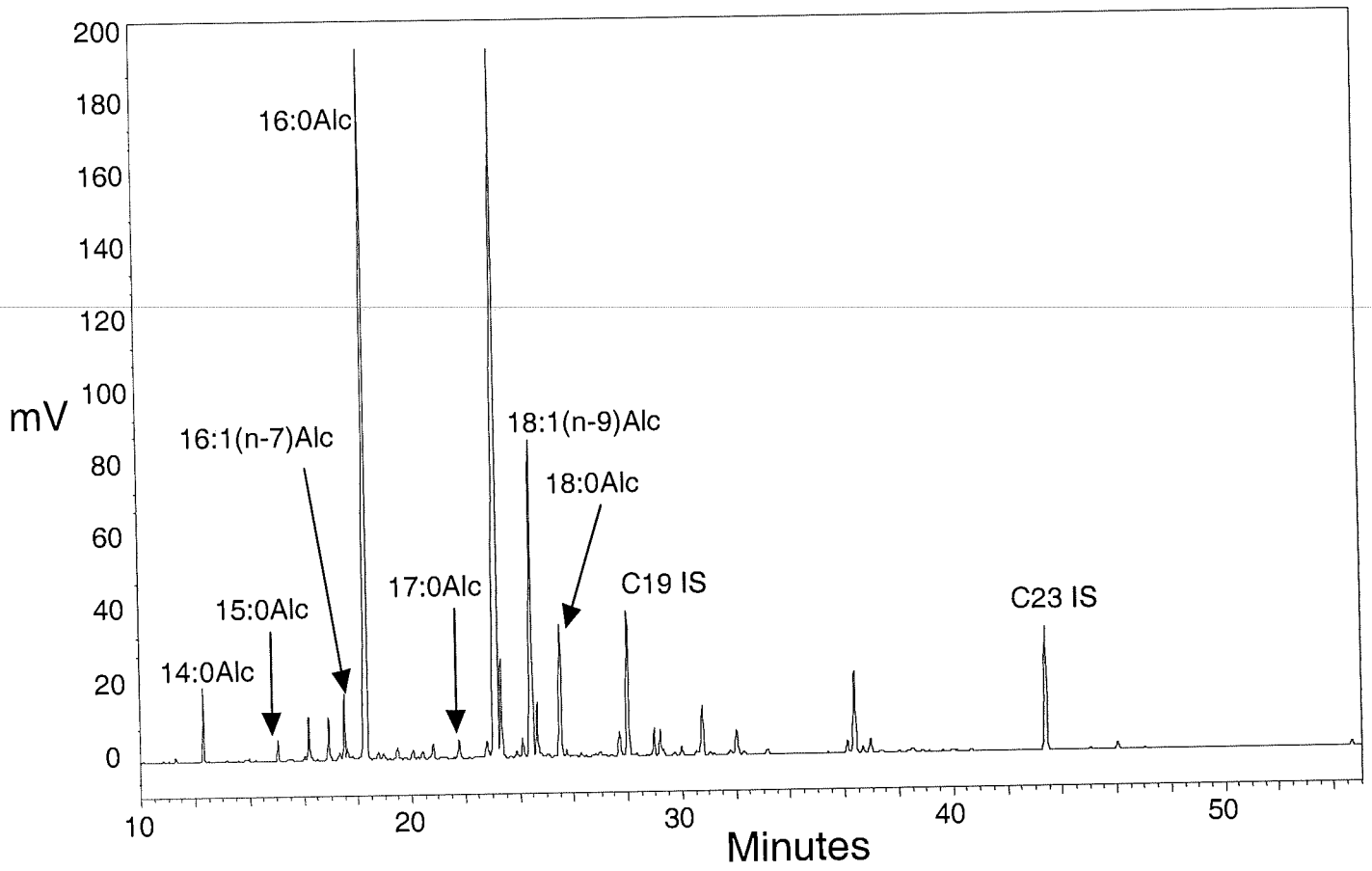


Figure 8.2. Partial gas chromatogram of fatty alcohols (as OTMSi ethers) derived from the wax ester-rich escolar *Lepidocybium flavobrunnen* (fatty alcohols obtained by transmethylation of total lipid). HP5 capillary column. Abbreviations: Alc, alcohol; IS, internal standard. Unlabelled peaks are fatty acid methyl esters.

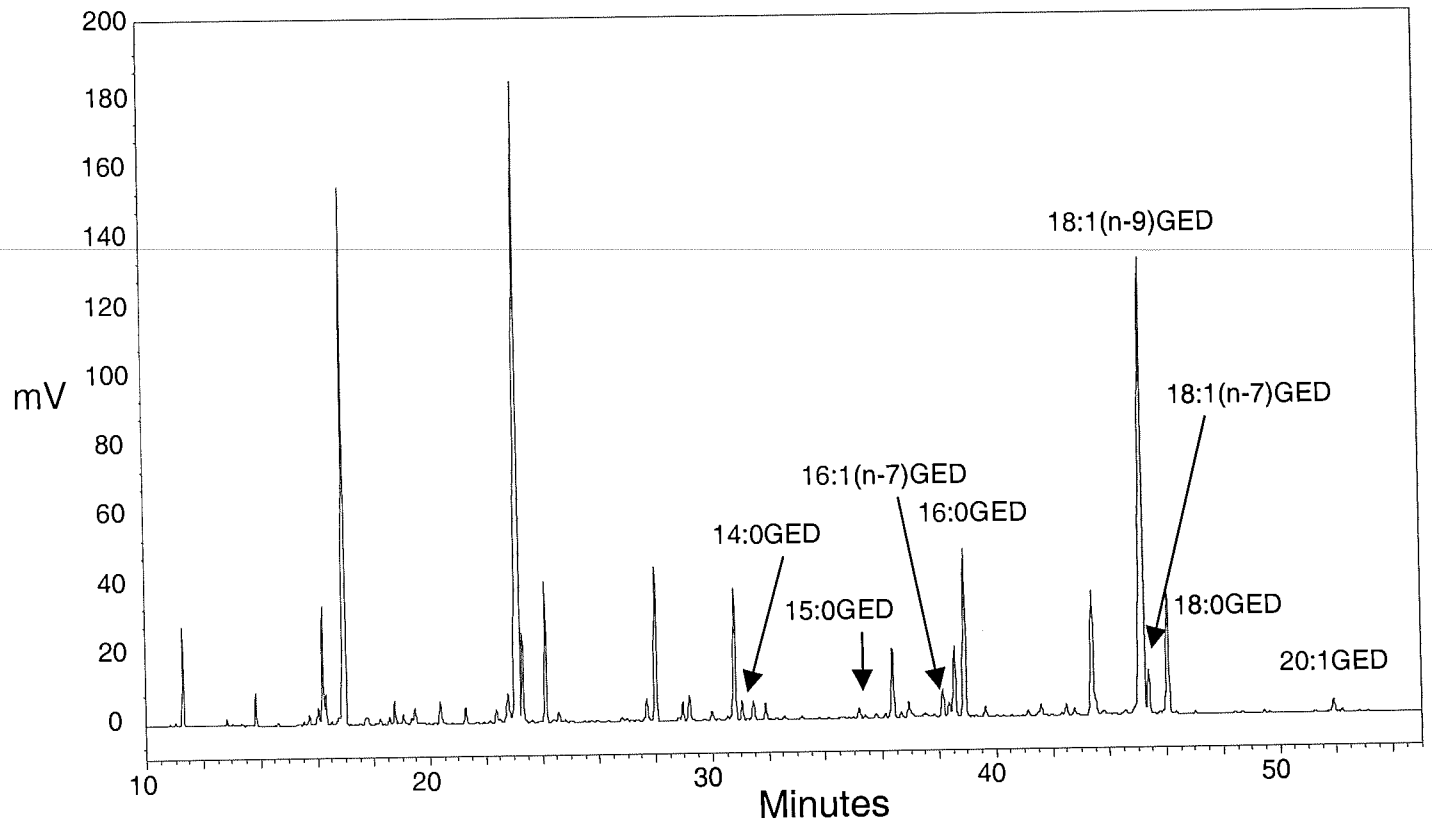


Figure 8.3. Partial gas chromatogram of non-saponifiable lipids of the rudderfish *Centrolophus niger*. The glyceryl ether diols (GED, as OTMSi ethers) are derived by transmethylation of the diacylglyceryl ethers, followed by silylation. HP5 capillary column. Abbreviations: Alc, alcohol; IS, internal standard; GED, glyceryl ether diol. Unlabelled peaks are fatty acid methyl esters and minor GED.

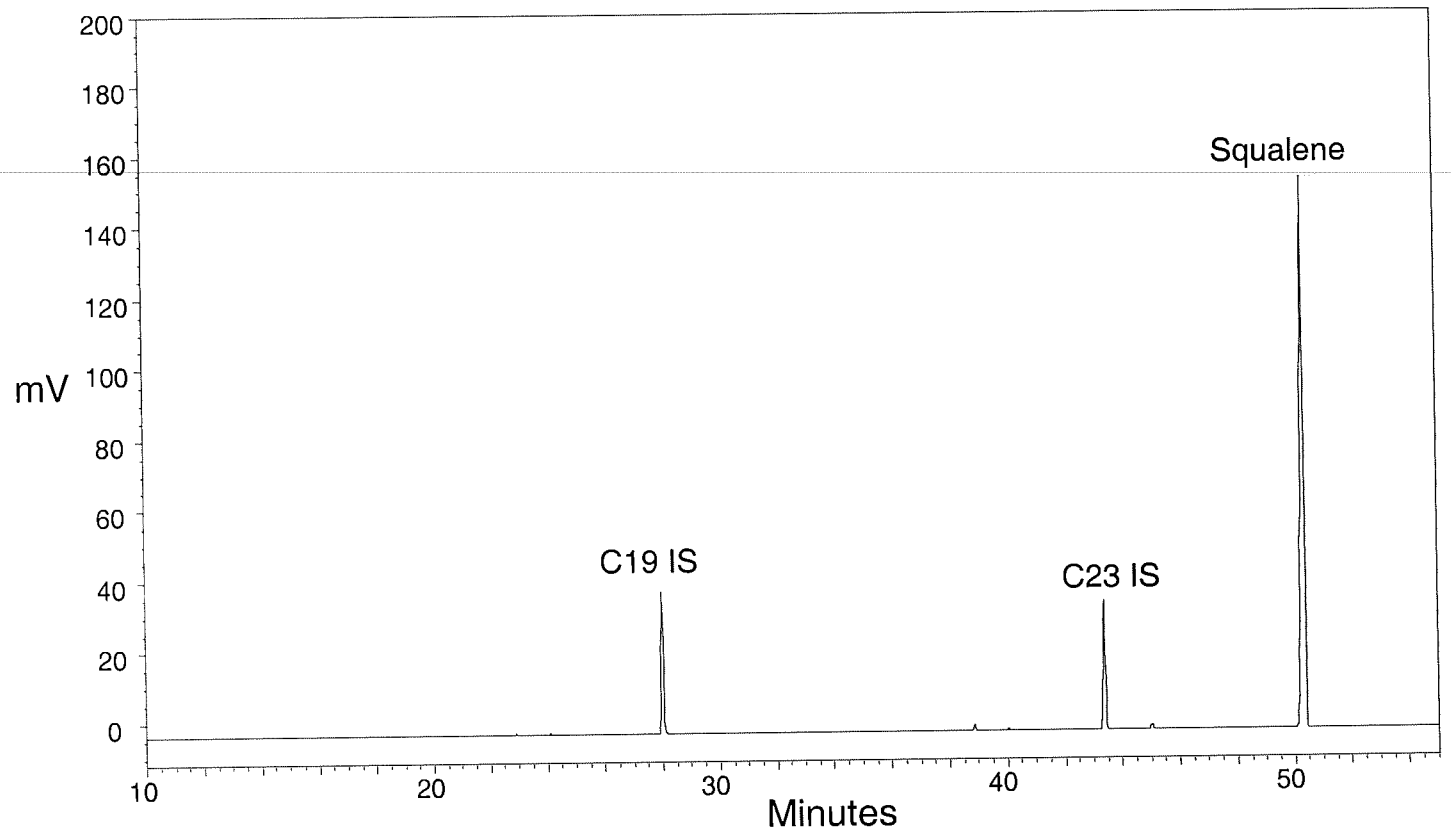


Figure 8.4. Partial gas chromatogram of non-saponifiable lipids of the rudderfish *Tubbia* spp. showing squalene as the dominate component. HP5 capillary column. Abbreviations: IS, internal standard.

9. Oil Content and Composition of Cooked Specimens of Purported Rudderfish

Research in this chapter was conducted for the Hunter Public Health Unit, NSW. Following circulation of the results outlined in chapter 8 during 2000, the project team was contacted and this additional research was approached on cooked purported rudderfish.

9.1 Introduction

The oil content and composition of cooked purported rudderfish samples were examined as part of FRDC Project 1999/331 (Chapter 8). Two cooked samples of fish (battered and deep-fried, and a sample of the cooking oil) were received from the Hunter Public Health Unit, NSW. The samples were reported to be the same type of fish, and had been cooked with the same method, to those causing illness in consumers. The symptoms of the illness were similar to those previously acknowledged from consumption of oil-rich fillets of the escolar marketing group; a group known to have unusual oil content and composition. Previous CSIRO research has shown that analysis of oil content and composition can distinguish between species of the escolar and rudderfish groups (Chapter 8). The earlier research was supported by genetic testing of the fillets.

9.2 Results and Discussion

The two unidentified specimens contained 22% oil, Table 9.1, a level well in excess of the average for Australian marine fish (1%) (Nichols *et al.* 1998 a and b), but consistent with that previously observed for individuals from the escolar and rudderfish groups (Nichols *et al.* 2001 and chapter 8). A further dissimilarity with most marine fish was that the oil composition was 96% and 98% wax ester (Table 9.1). This finding identifies the fillets as escolar rather than rudderfish (see Chapter 8). The cooking oil contained 83% triglyceride and contributed little to the oil content of the cooked fish. Our results demonstrate that the lipid profiling proceedures used in this study enable cooked specimens of escolar to be identified. This finding is noteworthy, as cooking may render other identification procedures ineffective.

These results are consistent with the two unknown samples originating from the escolar marketing group, and not rudderfish. The purgative effect of consuming escolar is noted in the national and international literature, and accounts for the reported illness in consumers (e.g. Yearsley *et al.* 1999; Mori *et al.* 1966, Cox and Reid 1932).

Table 9.1. Oil content and composition of cooked purported rudderfish. Data for known rudderfish and escolar samples are shown in Table 8.1.

Lipid Class	Sample 1	Sample 2	Cooking Oil
Wax ester	96.4	97.6	0.5
Triglyceride	1.9	0.3	82.6
Free fatty acid	*nd	nd	3.8
Unidentified	nd	nd	9.2
Polar lipid	1.7	2.1	3.9
Lipid content (% wet weight)	21.7	22.4	

*nd – not detected

10. The Good Oil – An Update on Tasmanian Atlantic Salmon

Presented as:

Nichols, P., Mooney, B. and Elliott, N. (2001) The good oil – an update on Tasmanian Atlantic salmon. In Battaglene, S. C. and Cobcroft, J. M. (eds.) Proceedings The First Scientific Conference of the Atlantic Salmon Aquaculture Subprogram. pp 63-65.

10.1 Introduction

There is increasing nutritional interest in the long-chain (C_{20} and C_{22}) omega-3 polyunsaturated fatty acids (LC-PUFA). In particular, eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3) in seafood and marine oil products receive special attention. This is the result of well-documented nutritional benefits of these unique LC-PUFA to humans and also farmed species. In humans, they help against coronary heart disease, high blood pressure, rheumatoid arthritis, and may also be beneficial against other disorders, including some forms of cancer, depression and other neural illnesses.

Most Australian seafood contains elevated levels of the long-chain omega-3 oils, including the beneficial DHA and EPA. The oil composition of 200 species of Australian seafood is available in the Guide *Seafood the Good Food* (Nichols *et al.* 1998a&b). Whilst considerable data now exists on the oil composition of Australian seafood, less information is available on aquaculture products, particularly on the effect of changing diet, and the various forms of value-adding, on the oil profiles. In this study we report on the effects of value-adding on the oil content and composition of Atlantic salmon, a premier aquaculture species farmed in pristine Tasmanian waters. This study was performed as part of a Fisheries Research and Development (FRDC) supported investigation of the oil composition of Australian Seafood.

10.2 Materials and Methods

Fish samples (generally 3 specimens per treatment) were provided by Tassal and Pivot. Approx. 5-100 g portions of fish were sampled. Modes of value-adding included smoking and dry-curing. The effects of changing the oil content of the diet were also assessed. All samples were placed in separate bags and stored frozen prior to analysis.

Oil (lipid) analyses were conducted using methods developed during FRDC projects 1995/122 and 1999/331. Full details are provided in Nichols *et al.* 1998a,b&c. Briefly, samples were extracted using a single phase Bligh and Dyer (1959) procedure. Oil yield was determined gravimetrically. An aliquot of the oil was analysed by TLC-FID to determine lipid class composition. Fatty acid profiles were obtained by capillary GC and GC-MS analysis following transmethylation of an aliquot of the extracted oil.

10.3 Results and Discussion

The average content of the beneficial omega-3 LC-PUFA in Australian fish is 210 mg/100 g, with slightly lower levels in shellfish and crustaceans (Table 10.1, Nichols *et al.* 1998a & b). Such levels are around tenfold or more greater than occurs in other food groups. In comparison, cultured fish fed marine oil based diets generally contain higher levels of the beneficial oils.

We have found that the oil and omega-3 LC-PUFA content of Atlantic salmon flesh can vary; levels of omega-3 LC-PUFA in fresh flesh were between 700 to 2300 mg/100 g of (Table 10.1). Such variation can be due to fish size, diet, genetic and/or environmental factors. Oil and omega-3 LC-PUFA content also may vary along the fillet, with highest levels recorded mid-fillet. High levels of oil and omega-3 LC-PUFA are found in lower-value cuts and body parts, with the highest values occurring in the belly-flap/viscera, followed by the frame and head. Some of these materials are used in the preparation of burger mince; these data indicate that the lower value portions may have higher nutritional value, in terms of omega-3 LC-PUFA content.

Smoked Atlantic salmon was found to have increased levels of oil and omega-3 LC-PUFA. No degradation of the PUFA occurred. The increase is largely due to a loss of water during the smoking process. In companion studies with other fish species, we have also examined the effect of various forms of cooking on oil and omega-3 LC-PUFA content. As with the analyses of value-added Atlantic salmon, the oil content of the fish varied depending on the method of cooking. With microwave or steam cooking, oil and omega-3 LC-PUFA content and composition were generally similar to fresh fish. Oil content of fish increased when fried or grilled. The higher oil content observed is consistent with uptake of cooking oil by the fillet, with the oil composition also influenced by the type of oil used - omega-6 PUFA and oleic acid increased when peanut and cottonseed oil were used respectively. Importantly, the content of the omega-3 LC-PUFA, mainly DHA and EPA, was largely not affected by any of the forms of cooking.

In summary, under the current feeding practices Tasmanian Atlantic salmon is an excellent source of the beneficial omega-3 LC-PUFA. Value-adding treatments and cooking do not diminish the omega-3 LC-PUFA content. Processing by-product ("waste") also offers unique opportunities for value-adding. Should fish meal and fish oil in current diets be replaced with other materials, the current high levels of omega-3 LC-PUFA may decrease, as may the nutritional value and potentially the product value.

Table 10.1. Comparison of omega-3 long-chain-polyunsaturated fatty acid (LC-PUFA) content of Tasmanian Atlantic salmon with other food groups.

Sample	Omega-3 LC-PUFA (mainly EPA+DHA) mg/100 g (wet weight)
Other food groups	
Turkey	35
Beef	22
Chicken	19
Lamb	18
Pork	0
Veal	0
Australian seafood *(wild, Nichols et al. 1998a&b)*	
Fish	210
Shellfish	150
Prawns	120
Lobster	105
Farmed Australian fish	
Silver perch	790
Striped perch	2480
Tasmanian Atlantic salmon *(farmed)*	
Whole fillet	1930
Skin	4050
Head	5370
Frame	5240
Belly flap/viscera	10270
Untreated	700
Cold smoked	1030
Hot smoked	840
Ishanabe (dry cured)	840
Low oil diet	
3 months	2130
6 months	1350
High oil diet	
3 months	2210
6 months	1420

EPA, eicosapentaenoic acid (20:5ω3); DHA docosahexaenoic acid (22:6ω3). Data shown for Australian seafood (wild) are average values from Nichols et al. (1998a&b).

11. The Oil Content and Composition of Atlantic Salmon: Effect of Dietary Oils

Results in this chapter were provided as a report to Pivot.
Elliott, N., Mooney, B. and Nichols, P. (2001) The oil content and composition of Atlantic salmon. Effect of dietary oils. Report 2001-Pivot.

11.1 Summary

- The oil (lipid) content and composition in the flesh of Atlantic salmon was examined as part of a PIVOT Aquaculture and Tassal flesh quality assessment trial of two feeds. Each feed was trialed in triplicate cages (total of six cages), and samples for oil analysis were provided in triplicate from each cage mid-way into the trial and at the end of the trial.

- The oil content of the two feeds, F22 and F33, had been designed to be 22% and 33%, respectively. Laboratory analyses indicated about a 10% increase in both, at 23.8% for F22, and 36.7% and 37.9% total oil for two samples of the F33 feed.

- The oil added to the F33 feed was changed during the trial period. The oil profile in the revised feed differed markedly from that in the original feed and that in the F22 fed, with an increased level of monounsaturated fatty acids (MUFA) and a decreased level of polyunsaturated fatty acids (PUFA). Individual component changes in this revised feed included an increase in 18:1ω9 and 22:1ω11, and decrease in the omega-3 EPA.

- The oil levels in the flesh of the Atlantic salmon decreased from ca. 8% to 5% under both feed regimes between the two sampling periods. These values are higher than reported for some other analyses, but are in the range observed for Tasmanian Atlantic salmon.

- Only small differences were observed in the levels of the main PUFA components, EPA and DHA, in the flesh of fish fed the different feeds or at the different sampling times. The oil and fatty acid profiles were very similar between all samples, with only minor differences apparent in levels of specific components. Individuals on the F33 feed had marginally higher levels of 18:1ω9 and 22:1ω11, possibly reflecting the profile of the revised feed. At the first sampling, the samples from the F33 feed also had marginally lower levels of EPA, but this was not apparent at the final sampling.

- The level of oil in the flesh of Atlantic salmon, from these results, is not influenced by the level in the feed when fed at a rate of 24% or 38% of the feed. Other environmental factors appear to have more influence on the oil content in the flesh.

11.2 Introduction

The acknowledgment of the benefit of marine oils, in particular the long-chain (C_{20} and C_{22}) omega-3 polyunsaturated fatty acids in human nutrition is increasing. The benefits of such components as eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3) include helping against coronary heart disease, high blood pressure and arthritis, as well as depression and other neural disorders. Eating regular meals of seafood and/or taking capsules containing marine oil (by-product from seafood) are continually being marketed as the best dietary sources of these essential fatty acids. Most Australian seafood has been shown to contain high levels of these nutritionally beneficial oils (Nichols et al 1998a&b), and Atlantic salmon with a natural high oil level has always been rated as a nutritionally valuable species. The amount of oil and therefore essential fatty acids in the flesh of cultured species has been shown to be dependent on environmental factors such as the level of oil in the feed, sexual maturity and season.

Flesh quality assessment trials in Atlantic salmon were being conducted by Pivot Aquaculture in association with Tassal. These trials involved the evaluation of two feeds. A range of quality assessment criteria were carried out by Pivot Aquaculture including such parameters as condition factor and colour scores. Of particular interest to Pivot Aquaculture and CSIRO, and in relation to the objectives of FRDC project 1999/331, was the fact that the two feeds differed in their lipid (oil) levels. These trials therefore provided an opportunity to assess the effects of increased oil levels in the feed on a species with naturally high oil levels in the flesh.

11.3 Materials and Methods

Samples

All samples of Atlantic salmon were collected and sampled by Pivot Aquaculture staff from stock held at the Tassal marine site at Nubeena. The samples were from fish stocked in commercial cages as smolts in late 1999 and designated for flesh quality assessment trials. Fish were harvested at random from the cages, and excluded maturing fish, runts, and those with deformities or in poor condition.

All fish were fed with Pivot Aquaculture's *Salmon Grower* 45/22 feed prior to the start of the trials. Trial cages were switched to the experimental diets in July 2000. Two feeds were assessed – F22 and F33. The experimental design by Pivot Aquaculture consisted of six (6) cages, with three (3) allocated per diet. Fish harvested from the cages at two occasions were provided for oil analyses – October 2000 and November/December 2000.

Samples provided to CSIRO consisted of muscle tissue from three (3) individuals per cage per time period, for a total of 36 samples.

Samples of each feed were also provided for analysis. The oil source for the F33 feed was changed in the middle of the trial (October) and therefore two samples (F33A and F33B) were analysed. The feed compositions were reported as:

F22 – Atlantic salmon grower 45/22 (45% Protein; 22% oil; 8.5% ash; 19.0 MJ digestible energy/kg feed; 64 mg astaxanthin/kg feed)
F33 – BioMar Ecolife salmon grower 40/33 (40% Protein; 33% oil; 6.7% ash; 22.4 MJ digestible energy/kg feed; 64 mg astaxanthin/kg feed).

Oil analyses

Oil (lipid) analyses were conducted using methods developed during FRDC projects 95/122 and 99/331. Full details are provided in reports prepared for FRDC (Nichols *et al.* 1998b and c), with the methods also published in the peer-reviewed literature (e.g. Bakes *et al.* 1995, Nichols *et al.* 1998a). Briefly, samples were extracted using a single phase Bligh and Dyer (1959) procedure. Oil yield was determined gravimetrically. An aliquot of the oil was analysed by TLC-FID to determine oil class composition. Fatty acid profiles were obtained by capillary GC and GC-MS analysis following transmethylation of an aliquot of the extracted oil.

11.4 Results and Discussion

Feeds
The oil level recorded in each of the feeds was close to 10% higher than specified. The analysis of the F22 feed returned a value of 23.8% total oil, while the F33 feeds were 36.7% and 37.9% total oil. Triglyceride was the dominant component in all feeds (90% to 95%), with minor proportions of phospholipids (Table 11.1).

The fatty acid profiles for the two feeds were very similar except for the revised F33 feed (F33B) used in the later part of the trial (Table 11.2). Polyunsaturated fatty acids (PUFA), in particular the long chain PUFA (LC-PUFA, $\geq C_{20}$) were dominant at 43% in the initial feeds, with EPA (20:5ω3) and DHA (22:6ω3) accounting for over half, at 15% and 13% respectively. The other main components were 16:0, 18:1ω9, 16:1ω7 and 14:0.

The revised F33B feed had a decreased level of monounsaturated fatty acids (MUFA) (25.7% compared to 43.2%) and an increased level of LC-PUFA (43.1% compared to 31.1%). Individual component changes in this revised feed included a decrease in 18:1ω9 (12.2% down from 21.1%) and 22:1ω11 (0.6% down from 5.7%), and increase in EPA (15.4% up from 7.5%). DHA levels were 12% to 13% in all feeds. The oil profile in the revised feed differed markedly from that in the original feed. Differences in the fatty acids reflected the change to a Northern Hemisphere originating oil.

Fish samples
In all samples the level of oil in the flesh decreased between the two sampling periods (Table 11.3). The mean level of oil in the flesh was about 8% at the first sampling (October), but decreased to approximately 5% at the final sampling (November/December). Triglyceride was the dominant component in all samples (80% to 92% means), with phospholipid at 7% to 18%. At the second sampling (Nov/Dec) a small decrease in the level of triglyceride was observed compared with the first samples (80% and 83% down from 92% and 91% respectively), with corresponding increase in phospholipid (7% and 8% up to18% and 15%).

The level of oil in the flesh of these samples was higher than that previously reported for Tasmanian Atlantic salmon at 2.7% (Nichols et al 1998b). Such variation is not unusual for

species with such high content, and as shown here can vary markedly both between individuals (high standard deviations about the means, Tables 11.3 and 11.4) and sampling times due to environmental influences. The change in oil level in the feed between 24% and 38%, however, does not have any major influence.

The high level of LC-PUFA in the feeds is reflected in the flesh (Table 11.4). Animals from the cages on the F33 feed had mean values of about 39% LC-PUFA at the both sampling times. Those on the F22 feed had values of about 42% at the first sampling in October and 40% at the second sampling in November/December (Table 11.4a). Very little difference in the levels of the main LC-PUFA components, EPA and DHA, was observed between feeds or sampling times. The fatty acid profiles were very similar between all samples, with only minor differences apparent in levels of specific components. These included individuals on the F33 feed having marginally higher levels of 18:1ω9 and 22:1ω11 compared with those on the F22 feed. At the first sampling, the individuals from the F33 feed also had marginally lower levels of EPA (mean of 8.7% compared to 10.4%), but this was not apparent at the second sampling (mean 9.9%) and reflects the original diet oil of these fish. DHA levels in the flesh were between 14% and 16%, with no variation between feeds or sampling time obvious. The fatty acid profile was similar to that previously observed (Nichols et al 1998b) for Tasmanian Atlantic salmon, although the relative level of EPA was marginally higher and that of AA (20:4ω6) and 16:0 slightly lower.

The absolute content (mg/100) of the main omega-3 LC-PUFA components, as with all other components, was highest at the first sampling in October (Table 11.4b, Figure 11.1); this finding is consistent with the increased oil content in the flesh. The mean omega-3 LC-PUFA level at the first sampling was around 2100 mg/100, compared to about 1400 mg/100 at the second sampling in November/December.

11.5 Conclusions

There was no evidence from this study of increased oil levels in the flesh of Atlantic salmon with exposure to a feed with higher oil levels (38% compared to 24%). Oil levels recorded were in the range previously recorded for Tasmanian Atlantic salmon. The oil content in the flesh would appear to be more influenced by conditions other than levels in the feed.

The oil profile was similar to that previously observed for Tasmanian Atlantic salmon and did not differ markedly between individuals on the different feeds.

To be able to eliminate extraneous environmental factors in future experiments it is recommended that either individual fish are monitored over time through use of biopsy sampling and/or individuals from the same family (reduced genetic variation component) are monitored.

11.6 Acknowledgment

We thank Tassal for assistance in the field and access to stock at their Nubeena site for the feed trials conducted by Pivot Aquaculture.

Table 11.1. Pivot feeds. Proportion of oil classes, presented as content (mg/100g) and composition (%). Feed F33A used up to October 2000, and then replaced by F33B. The F22 feed was used throughout the experiment.

	Feed		
	F22	**F33A**	**F33B**
Content (mg/100g)			
Triglyceride	217.2	348.2	340.7
Free fatty acid	3.5	2.7	7.6
Cholesterol	1.8	1.6	3.7
Phospholipid	15.0	14.1	27.5
Total	237.5	366.7	379.4
% Total oil	**23.8**	**36.7**	**37.9**
Composition (%)			
Triglyceride	91.5	95.0	89.8
Free fatty acid	1.5	0.7	2.0
Cholesterol	0.8	0.4	1.0
Phospholipid	6.3	3.9	7.2

Table 11.2. Pivot feeds. Composition (%) of main fatty acids. SFA – total saturated fatty acids; MUFA – total monounsaturated fatty acids; PUFA – total polyunsaturated fatty acids.

	Feed		
	F22	**F33A**	**F33B**
14:0	6	4	7
C16PUFA	3	1	4
16:1ω7c	7	5	7
16:0	19	16	19
18:4ω3	3	3	2
18:2ω6	3	4	2
18:1ω9c	12	21	12
18:1ω7c	3	2	3
18:0	4	3	4
20:4ω6 AA	1	1	1
20:5ω3 EPA	14	8	15
20:1ω9c	1	4	1
22:6ω3 DHA	13	12	13
22:5ω3	2	1	2
22:1ω11	1	6	1
24:1	1	2	1
other	7	8	6
Total SFA	32	26	31
Total MUFA	26	43	26
Total PUFA	43	31	43
Total omega-3	33	24	34
Total omega-6	5	6	4

Other includes: 12:0, 14:1, i15:0, a15:0, 15:0, i16:0, 16:1ω9, 16:2, 16:1ω5, i17:0, 17:1ω8, a17:0, 17:0, 18:3ω6, 18:3ω3, 18:2, 18:1ω5c, 20:3ω6, 20:4ω3, 20:2ω6, 20:1ω11, 20:1ω7, 20:0, 21PUFA, 22:5ω6, 22:4ω6, 22:1ω9, 22:1, 22:0, 23PUFA, 24:0

Table 11.3. Atlantic salmon. Oil class content (mg/100g) and composition (%) in the flesh at two time points (October and November/December) on the two feeds (F22 and F33). Mean and standard deviation (SD) are presented for 3 individual fish at each sampling time and feed.

	F22		F33	
	Oct 2000	**Nov/Dec 2000**	**Oct 2000**	**Nov/Dec 2000**
Content (mg/100g)	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD
Triglyceride	7743.3 ±279.4	4656.4 ±2103.4	6593.2 ±1417.2	4205.4 ±449.3
Free fatty acid	82.5 ±11.6	70.5 ±29.8	75.4 ±23.9	63.1 ±17.8
Cholesterol	26.9 ±7.3	39.6 ±10.5	28.4 ±10.4	35.5 ±7.6
Phospholipid	552.3 ±78.4	756.7 ±114.4	582.6 ±114.3	636.2 ±111.8
Total	8405.1 ±317.3	5523.2 ±2083.0	7279.7 ±1361.8	4940.1 ±460.2
% Total oil	**8.4 ±0.3**	**5.5 ±2.1**	**7.3 ±1.4**	**4.9 ±0.5**
Composition (%)				
Triglyceride	92.1 ±0.8	80.0 ±5.9	90.6 ±3.4	83.1 ±2.6
Free fatty acid	1.0 ±0.2	1.5 ±0.5	1.0 ±0.1	1.4 ±0.3
Cholesterol	0.3 ±0.1	0.9 ±0.2	0.4 ±0.1	0.8 ±0.1
Phospholipid	6.6 ±0.9	17.6 ±5.8	8.0 ±3.5	14.7 ±2.4

Table 11.4. Atlantic salmon. a. major fatty acid composition (%) and b. content (mg/100g) in the flesh at two time points (October and November/December) on the two feeds (F22 and F33). Mean and standard deviation (SD) are presented for 3 individual fish at each sampling time and feed.

Table 11.4a. Fatty acid composition (%)

	F22		F33	
	Oct 2000	**Nov/Dec 2000**	**Oct 2000**	**Nov/Dec 2000**
	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD
14:0	4.5 ±0.6	4.8 ±0.3	4.2 ±0.4	5.0 ±0.3
C16PUFA	1.0 ±0.1	1.0 ±0.1	0.7 ±0.1	0.8 ±0.1
i16:0	0.8 ±0.1	0.8 ±0.1	0.6 ±0.1	0.8 ±0.0
16:1ω9c	0.3 ±0.0	0.2 ±0.0	0.3 ±0.0	0.3 ±0.0
16:1ω7c	6.3 ±0.4	6.7 ±0.4	5.9 ±0.3	6.5 ±0.3
16:2	0.8 ±0.1	0.8 ±0.1	0.7 ±0.0	0.8 ±0.1
16:0	17.1 ±0.5	18.8 ±0.3	16.5 ±0.5	17.9 ±0.6
17:0	0.5 ±0.0	0.6 ±0.1	0.5 ±0.1	0.5 ±0.1
18:4ω3	2.8 ±0.1	2.7 ±0.1	2.6 ±0.1	2.7 ±0.1
18:2ω6	3.2 ±0.2	3.1 ±0.1	3.7 ±0.1	3.3 ±0.2
18:1ω9c	15.8 ±1.1	16.0 ±0.7	18.4 ±0.3	16.6 ±0.9
18:1ω7c	3.2 ±0.1	3.4 ±0.0	3.2 ±0.1	3.3 ±0.1
18:0	4.2 ±0.2	4.4 ±0.2	3.7 ±0.1	4.0 ±0.2
20:4ω6 AA	0.9 ±0.0	1.1 ±0.1	0.9 ±0.0	1.0 ±0.1
20:5ω3 EPA	10.4 ±0.7	10.2 ±0.4	8.7 ±0.2	9.7 ±0.6
20:4ω3	1.5 ±0.0	1.4 ±0.0	1.5 ±0.1	1.5 ±0.0
20:1ω9c	1.7 ±0.2	1.3 ±0.2	2.6 ±0.1	1.8 ±0.3
22:6ω3 DHA	15.6 ±1.4	14.2 ±1.4	14.4 ±1.1	14.2 ±1.3
22:5ω3	3.7 ±0.3	3.1 ±0.1	3.3 ±0.3	3.1 ±0.1
22:1ω11	0.5 ±0.2	0.4 ±0.1	1.8 ±0.3	0.9 ±0.2
24:1	0.5 ±0.1	0.3 ±0.0	0.7 ±0.1	0.4 ±0.0
other	4.8	4.7	5.1	4.9
Total	**6.4 ±1.6**	**4.3 ±1.1**	**7.3 ±0.9**	**4.6 ±1.9**
Total SFA	28.2 ±1.1	30.5 ±0.5	26.7 ±1.0	29.3 ±0.9
Total MUFA	30.0 ±1.5	29.9 ±1.1	35.0 ±0.5	31.5 ±1.6
Total PUFA	42.0 ±1.9	39.8 ±1.0	38.5 ±1.3	39.3 ±1.4
Total omega-3	34.0 ±2.1	31.6 ±1.1	30.5 ±1.4	31.1 ±1.5
Total omega-6	5.4 ±0.1	5.2 ±0.2	5.8 ±0.1	5.4 ±0.2

Table 11.4b. Fatty acid content (mg/100 g)

	F22		F33	
	Oct 2000	**Nov/Dec 2000**	**Oct 2000**	**Nov/Dec 2000**
	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD
14:0	296.2 ±103.5	209.8 ±63.3	306.6 ±72.5	230.5 ±101.3
C16PUFA	62.3 ±19.5	42.1 ±13.9	51.6 ±12.1	39.2 ±16.9
i16:0	52.2 ±15.9	37.2 ±12.0	47.0 ±10.4	36.1 ±15.5
16:1ω7c	405.4 ±119.8	293.7 ±88.1	431.9 ±84.0	303.7 ±131.7
16:2	50.7 ±14.9	37.0 ±11.1	52.3 ±9.8	37.2 ±19.0
16:0	1096.3 ±295.5	813.9 ±205.9	1206.6 ±192.3	824.6 ±324.3
17:0	32.9 ±10.5	25.5 ±8.0	35.4 ±9.2	25.0 ±10.5
18:4ω3	180.4 ±50.0	118.5 ±34.9	187.0 ±31.8	124.9 ±54.3
18:2ω6	208.9 ±56.1	134.8 ±34.5	269.0 ±38.4	155.4 ±70.3
18:1ω9c	1021.4 ±282.0	699.1 ±180.6	1344.4 ±201.5	777.4 ±355.8
18:1ω7c	207.5 ±53.3	147.3 ±36.9	230.4 ±37.4	151.5 ±64.0
18:0	262.4 ±61.3	190.8 ±46.7	270.3 ±36.4	181.7 ±66.7
20:4ω6 AA	57.6 ±13.9	45.3 ±9.8	64.0 ±8.7	46.5 ±16.4
20:5ω3 EPA	654.3 ±145.1	438.9 ±121.7	635.2 ±83.3	444.1 ±173.2
20:4ω3	95.4 ±22.4	59.3 ±14.0	109.4 ±14.2	67.1 ±28.7
20:1ω9c	107.6 ±30.8	58.1 ±14.3	186.6 ±22.0	85.0 ±44.2
22:6ω3 DHA	959.8 ±159.7	597.6 ±102.0	1045.8 ±84.6	636.3 ±197.9
22:5ω3	233.0 ±41.8	135.2 ±35.2	237.4 ±23.7	143.0 ±60.4
22:1ω11	35.2 ±17.5	16.5 ±6.8	128.0 ±23.4	43.9 ±26.3
24:1	30.9 ±4.2	14.4 ±3.8	50.5 ±11.1	17.8 ±6.8
other	329.6	215.4	398.6	239.3
Total	**6379.9 ±1584.2**	**4330.4 ±1079.3**	**7287.8 ±926.4**	**4610.1 ±1883.9**
Total SFA	1777.5 ±495.9	1298.8 ±339.7	1915.3 ±330.8	1326.7 ±530.4
Total MUFA	1906.3 ±526.7	1297.3 ±334.0	2498.9 ±372.4	1459.2 ±660.6
Total PUFA	2655.0 ±554.1	1717.1 ±397.8	2810.4 ±316.9	1804.2 ±676.9
Total omega-3	2125.6 ±417.6	1349.5 ±304.2	2214.8 ±219.3	1415.4 ±513.2
Total omega-6	343.2 ±86.9	225.1 ±56.2	420.9 ±56.8	250.6 ±107.8

Other includes: 12:0, 14:1, i15:0, a15:0, 15:0, 16:1ω9, 16:1ω5, i17:0, 17:1ω8, a17:0, 18:3ω6, 18:3ω3, 18:2, 18:1ω5c, 20:3ω6, 20:2ω6 20:1ω11, 20:1ω7, 20:0, 21PUFA, 22:5ω6, 22:4ω6, 22:3ω3, 22:1ω9, 22:1ω7, 22:0, 23PUFA, 24:0

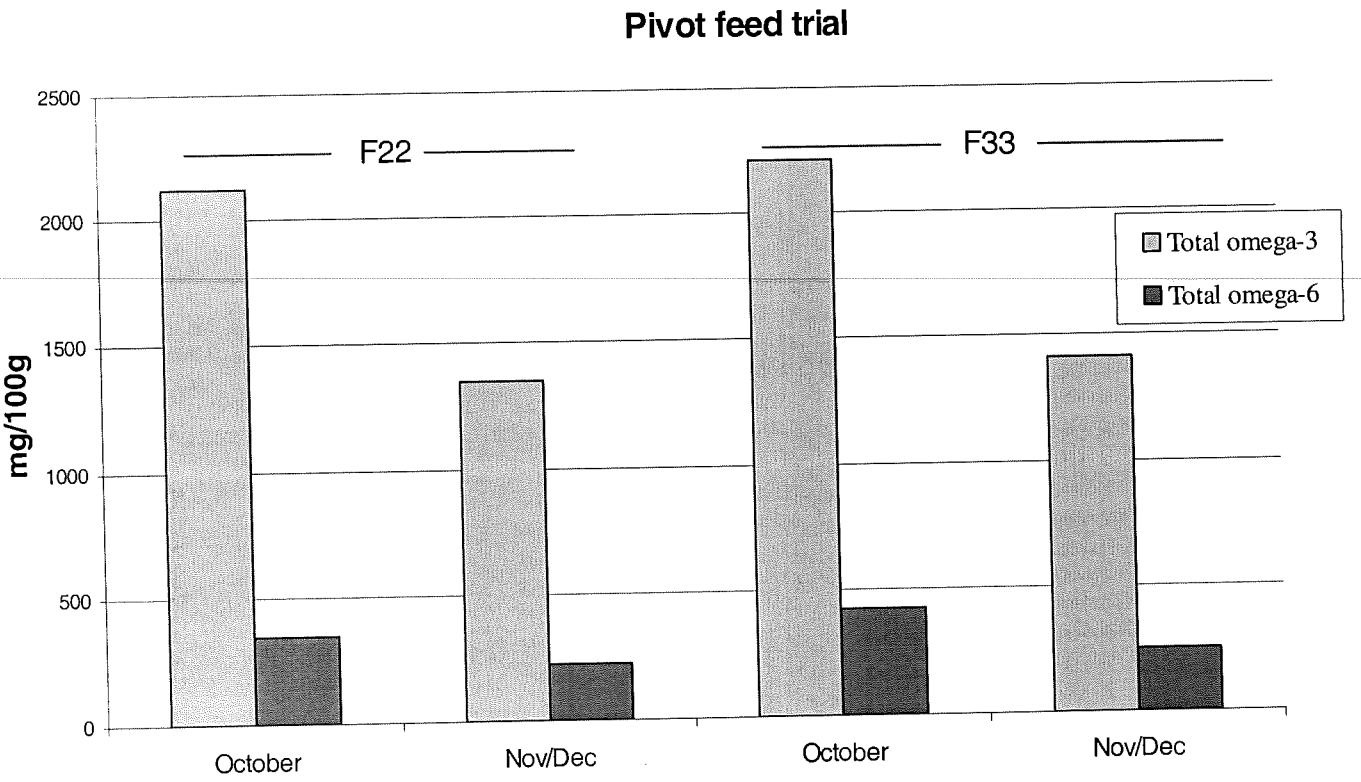


Figure 11. 1. Atlantic salmon. Comparison of total omega-3 and omega-6 PUFA content (mg/ 100 g) of individuals on two feeds (F22 and F33) at two sampling times (October and November/December 2000).

12. The Effect of Processing on the Oil Content and Composition of Tasmanian Atlantic Salmon

Results in this chapter were provided as a report to Tassal.
Mooney, B., Elliott, N. and Nichols, P. (2002) The effect of processing on the oil content and composition of Tasmanian Atlantic salmon. Report 2002-Tassal1.

12.1 Summary

- Aquacultured Atlantic salmon were examined to determine the effect of different processing procedures on oil content and composition of the fillets. Emphasis was placed on the levels of the beneficial long-chain (C_{20} and C_{22}) omega-3 polyunsaturated fatty acids (LC-PUFA), in particular eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3).

- Processing methods were: cold smoking, hot smoking and salting (termed Ishanabe). The effects of salting and storing of caviar were also examined. The by-product after filleting was examined, as well as minced product for burger mince.

- A standardised sample from the right shoulder region of Atlantic salmon varied in oil content from 1.5 to 3.7% in individuals prior to processing. Samples analysed along the fillet (transverse) ranged from 2.2 to 7.6%, with the highest oil content in the middle, tapering off towards the head and tail. The omega-3 LC-PUFA, predominantly DHA and EPA, accounted for 40-47% (370-1220 mg/100 g) of the total fatty acids in fresh fish (right shoulder). DHA was the dominant component at 22-29%.

- The by-product (gut, skin, frame, trim, head) component of Atlantic salmon contained high levels of oil and omega-3 LC-PUFA. The by-product contained between 14 and 36% oil, with LC-PUFA ranging from 4050 to 10300 mg/100 g. These very high contents highlight the potential for the by-product stream to be utilised in value-added products, e.g. fish oil capsules.

- Changes in oil content of the fillets of Atlantic salmon varied depending on the method of processing. With cold and hot smoking, oil content and omega-3 LC PUFA generally increased, consistent with water loss during smoking. Ishanabe treatment caused a decrease in the oil content from 3.3 to 2.3%, with an increase in water content. We note the content of the omega-3 LC-PUFA, DHA and EPA, was largely not affected by these forms of processing.

- Commercially available salmon burgers derived partly from salmon by-products were examined for oil content and composition. Oil content of the fresh salmon mince and commercial burger product were 25% and 10% respectively. The LC-PUFA DHA, EPA and AA accounted for 31% of the total fatty acids in fresh and 14% in the commercial burger preparation. On an absolute basis, the omega-3 LC-PUFA content was – fresh salmon mince, 6400 mg/100 g, and commercial burger 1800 mg/100 g. These values are considerably higher than the average value observed for Australian seafood.

12.2 Introduction

Interest is increasing in the nutritional benefits of the long-chain omega-3 (C_{20} and C_{22}) polyunsaturated fatty acids (LC-PUFA). Eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3) in seafood and marine oil products receive special attention as a result of well-documented nutritional benefits of these unique PUFA. They help against coronary heart disease, high blood pressure, rheumatoid arthritis, and may also be beneficial against other disorders, including some forms of cancer, depression and other neural illnesses. Most Australian seafood contains elevated levels of the long-chain omega-3 oils, including the beneficial DHA and EPA. The oil composition of 200 species of Australian seafood is available in the Guide *Seafood the Good Food* and in an accompanying FRDC report (Nichols *et al.* 1998a&b).

Whilst considerable data now exists on the oil composition of Australian seafood, knowledge on the effect of processing, including smoking and other forms of value-adding, on the oil profiles, is limited. There are numerous reports on the lipid composition and content of Atlantic salmon (e.g. Katikou *et al.* 2001, and references cited therein). The distribution of lipids in the muscle varies with the origin of the fish, season and individual, and part of the muscle. This study presents data on the effects of processing on the same fillet, with samples taken in the same region, both before and after processing. In this way we can assess the effect of the processing methods on the individual fillet, regardless of genetic and environmental factors.

We report on the effects of cold smoking, hot smoking, and Ishanabe (salting) on oil content and composition in fillets, as well as the effect of salting and storing roe (caviar). Lipid and fatty acid profiles were obtained also for by-products from filleting (mince, skin, head, frame, viscera, trim). In addition, a transverse section along the fillet was examined to investigate the change in oil content and composition along the fillet.

This study was performed as part of a Fisheries Research and Development Corporation (FRDC) supported investigation (1999/331) of the oil composition of Australian Seafood.

12.3 Materials and Methods

Samples

(i) Hot and Cold Smoking and Ishanabe

Three right hand side fillets were examined for each of the three Tassal commercial processes. Fresh fillets were tagged and a small tissue sample (~3g) taken from the shoulder region and mid fillet (Figure 12.1). After processing, tissue samples were taken from the same two positions on each fillet. Samples were stored at -20^0C prior to analysis.

Cold smoking (sampled $6^{th}/7^{th}$ August 2000) involved overnight smoking at 27^0C.
Hot smoking (sampled $29^{th}/30^{th}$ August 2000) involved overnight smoking at 70-80^0C.
Ishanabe (sampled $6^{th}/7^{th}$ August 2000) involved brine treatment overnight at 27^0C.

(ii) By-product

Samples were obtained, in triplicate, from the Tassal processing plant at Huonville. Samples of skin, frame, head, viscera, burger mince and trimmings were taken during the normal filleting procedure on 7th July 2000. Samples were from different fish. The burger patties (from burger mince) were obtained from Tassal Huonville, and were prepared by Colonial Farms.

(iii) Whole fillet and along the fillet

A whole fish was obtained from Tassal Huonville on 7th July 2000. The fish was filleted and seven samples (~3g) taken at evenly spaced intervals along the dorsal region of the right hand-side fillet (Figure 12.2). The left hand-side fillet was homogenisd using a Barmix, subsampled in triplicate and analysed.

(iv) Caviar

Samples of caviar were taken from the Tassal processing plant at Dover on 20th February 2001. Samples, obtained in triplicate, were fresh and unwashed caviar, salted and washed caviar, and also caviar stored in a glass jar for twelve months (at 4^0C).

Oil analyses

Oil (lipid) analyses were conducted using methods developed during FRDC projects 1995/122 and 1999/331. Full details are provided in reports prepared for FRDC (Nichols *et al.* 1998a&b), with the methods also published in the peer-reviewed literature (e.g. Bakes *et al.* 1995, Nichols *et al.* 1998c). Briefly, samples were extracted using a single phase Bligh and Dyer (1959) procedure. Oil yield was determined gravimetrically. An aliquot of the oil was analysed by TLC-FID to determine lipid class composition. Fatty acid profiles were obtained by capillary GC and GC-MS analysis following transmethylation of an aliquot of the extracted oil.

12.4 Results and Discussion

Fresh samples

The lipid content in the fresh tissue of the nine fillets used in the study showed typical variation for Atlantic salmon. Right shoulder samples ranged in oil content from 1.5 to 3.7%, with a mean of 2.4% (Tables 12.1, 12.4 and 12.6). Mid fillet samples ranged in content from 1.0 to 4.6%, with a mean of 2.1% (Tables 12.2, 12.5 and 12.7).

The oil class and fatty acid profiles are generally similar to those of Tasmanian Atlantic salmon reported in *Seafood the Good Food*, although much higher levels of DHA and omega-3 LC-PUFA occurred in this study. These differences may be due to variations in feed composition, environmental, genetic and other factors.

Cold smoking

Lipid class, total lipid and fatty acid content are presented for three individual fillets before and after cold smoking (Table 12.1 – right shoulder samples, Table 12.2 – mid fillet samples). Total lipid increased in shoulder samples in two of the three fillets (Table 12.1), and in all three samples from the middle of the fillet (Table 12.2). This finding is consistent with water loss from the fillet during processing. The right shoulder fresh Sample 3 (Table 12.1) contained much higher levels of triglyceride (66%) and decreased in oil content after smoking. Major lipid classes in all samples were triglyceride (14-74%) and polar oil (32-80%), with lower levels of cholesterol and free fatty acids. The low levels of free fatty acids (0.3-1.1%) in all smoked samples is consistent with little degradation of lipid occurring due to heat/oxidation. There was little consistency in lipid class composition between individuals.

Fatty acid content increased with cold smoking in Samples 1 and 2, with a decrease in Sample 3, on the right shoulder, and increased in all mid fillet samples. Levels of the beneficial omega-3 LC-PUFA were between 500-1100 mg/100 g in fresh, and 700-1600 mg/100 g in cold smoked samples. This value for fresh salmon is 2-5 times higher than a previously reported average for Australian fresh fish (210 mg/100 g; Nichols *et al.* 1998b&c); and 4 to 8 times higher for cold smoked product. There was little change in the relative levels of fatty acids in cold smoked samples compared to fresh samples (Table 12.3). DHA was the dominant component at 27-29%.

Hot smoking

There was an increase in total oil and omega-3 LC-PUFA content in all samples following hot smoking (Table 12.4 and 12.5). The greatest increase was in Sample 3, right shoulder, from 1.6 to 4.0% (Table12. 4). Major lipid classes in all samples were triglyceride (13-84%) and polar oil (15-81%), with lower levels of cholesterol and free fatty acids. Again little degradation was observed with low levels of free fatty acid (0.2-1.3%). In all samples, the relative levels of triglyceride increased with hot smoking, with a corresponding decrease in polar oil. The omega-3 LC-PUFA content was 400-700mg/100 g in fresh samples, and 700-1300 mg/100 g after smoking. Hot smoked Tasmanian Atlantic salmon represents an excellent source of the omega-3 fatty acids. There was little change in the relative levels of fatty acids in hot smoked samples compared to fresh samples (Table 12.3). DHA was the dominant component at 20-27%.

Ishanabe

Total lipid decreased in all samples from the right shoulder (Table 12.6) and for Samples 2 and 3 in the mid fillet (Table 12.7). Sample 1 (mid fillet, Table 12.7) increased from 2.9% to 3.4%. The lipid class profile was dominated by triglyceride (28%-79%) and polar oil (20%-71%). Omega-3 LC-PUFA also decreased in all samples (except Sample 1, mid fillet, Table 12.7), from 2000-4300 mg/100 g in fresh salmon, to 1300-3000 mg/100 g in treated salmon. There was little change in the relative levels of fatty acids in Ishanabe samples compared to fresh samples (Table 12.3). DHA was the dominant component at 22-23%.

Whole fillet and along the fillet

The oil content along the fillet peaked in the middle (Sites 3 and 4, Figure 12.2) and tapered off at the ends (Table 12.8, Figure 12.3). The average for an homogenised sample from the whole fillet was also high (6.7%), nearly three times greater than the oil content reported for right shoulder samples of Tasmanian Atlantic salmon in Seafood the Good Food (2.7%), and the standard shoulder region samples in this study (2.4%). High levels of polar oil were present at the shoulder (Site 1) of the fillet, with the remaining area of the fillet dominated by triglyceride (85-90%, Table 12.8). The LC-PUFA, mainly EPA and DHA, were 32-45% of total fatty acids (Table 12.9). Higher levels of EPA (11%) and DHA (28%) were present in the sample taken closest to the head (Site 1). This is consistent with the occurrence of greater polar oil in this sample.

By-products

The by-product of the filleting process of Atlantic salmon contained very high levels of oil compared with the fillet (Table 12.11, Figure 12.4). The viscera (gut) contained the highest oil with 36%, then trim (23%), head (20%), frame (18%) and skin (14%). The fresh salmon mince contained 25% oil, and the commercial burger 10% (Figure 12.5). The oil profile in all samples was dominated by triglyceride (78-97%), with lower levels of polar oil, cholesterol and free fatty acid. The LC-PUFA accounted for 38-49% of the total fatty acids in the by-product stream, again dominated by EPA and DHA (Table 12.12). On a weight basis, the omega-3 LC-PUFA content was between 4000-10300 mg/100 g (Table 12.13, Figure 12.4). Relative to other food groups including seafood, these are extremely high levels of the beneficial LC-PUFA, and represent an excellent source of oil for the human health market and potential incorporation into functional foods. The fresh salmon mince contained a very high content of omega-3 LC-PUFA (6400 mg/100 g), with less in the final burger (1800 mg/100 g), which had higher levels of vegetable oil-derived fatty acids 18:2ω6 (1300 mg/100 g) and 18:1w9 (3600 mg/100 g).

Caviar

The oil content of Atlantic salmon caviar was 10% (unsalted), 15% (salted) and 11% (salted and stored). The oil classes were dominated by triglyceride (44-55%) and polar oil (43-54%) in all caviar samples (Table 12.14).

The fatty acid profile of caviar was very similar to that of the fillet, with EPA and DHA the most abundant components (Table 12.15). Little effect on the oil and fatty acid composition and content of the caviar was observed with salting and storage. The content of the omega-3 LC-PUFA in all samples of caviar was high, between 2600-3700 mg/100 g, and represents an extremely good source of these oils.

12.5 Conclusion

The current study represents, to our knowledge, one of the first detailed comparative studies of the effect of various value-adding processes and preservation methods on the oil content and composition of aquacultured Atlantic salmon. Although there was considerable variation between and along fillets, strict sampling protocol was used to overcome these effects and provided important nutritional information on the effect of value-adding seafood.

Importantly no loss of the beneficial omega-3 LC-PUFA was observed for any of the forms of processing examined. An increase in oil content was seen with cold and hot smoking, reflecting water loss from the flesh. The by-product from processing contained high levels of oil and omega-3 LC-PUFA, and represent a valuable source of oil for value-adding and possible refining for human nutraceuticals. Salmon caviar is also an excellent source of omega-3 LC-PUFA, and there was no loss of these components from caviar by salting or storing.

The fresh salmon mince contained much higher levels of the beneficial omega-3 LC-PUFA than in the fillet. In terms of nutritional value, the ratio of omega-3 to omega-6 fatty acids decreased in the commercial burger. Such changes in oil profiles will need further nutritional evaluation. The use of vegetable oils containing lower levels of omega-6 PUFA in the production of patties may also be worthy of consideration.

Our whole fillet and along fillet sample analyses of Atlantic salmon demonstrate that our standard right shoulder sampling tends to under-state the level of oil in the fillet and over-state the relative proportion of PUFA, such as DHA. Neither takes away from the fact that Atlantic salmon, fresh or processed, is a high oil fish with high levels of beneficial PUFA.

12.6 Acknowledgment

Tassal kindly provided fish, fish by-product, and caviar analysed in this study. We thank Cindy Hope and Alex Brettingham-Moore for their help with sampling, and, together with Pheroze Jungawalla, for their ongoing interest in and support of the project. Mark Rayner provided useful comments on smoking and preserving fish as well as contributing to the taste panel.

Table 12.1. Lipid class and fatty acid composition of fresh and cold smoked Atlantic salmon - right shoulder

Lipid class	*Sample 1*		*Sample 2*		*Sample 3*	
	Fresh	Smoked	Fresh	Smoked	Fresh	Smoked
mg/100 g						
Triglyceride	412	358	1245	1954	2152	316
Free fatty	6	17	5	5	6	17
Cholesterol	15	22	16	21	16	17
Polar oil	1081	1359	1307	1430	1090	1376
Total	1515	1755	2573	3410	3264	1726
% Total lipid	1.5	1.8	2.6	3.4	3.3	1.7
% composition						
Triglyceride	27.2	20.4	48.4	57.3	65.9	18.3
Free fatty	0.4	1.0	0.2	0.1	0.2	1.0
Cholesterol	1.0	1.2	0.6	0.6	0.5	1.0
Polar oil	71.4	77.4	50.8	42.0	33.4	79.7
Fatty acid	*Sample 1*		*Sample 2*		*Sample 3*	
	Fresh	Smoked	Fresh	Smoked	Fresh	Smoked
mg/100 g						
14:0	33	35	80	125	106	24
16:1ω7	41	44	91	141	140	34
16:0	242	297	376	529	452	245
18:4ω3	16	16	34	58	49	13
18:2ω6	20	25	42	75	63	26
18:1ω9	115	141	242	371	366	124
18:1ω7	31	42	58	90	77	34
18:0	58	70	91	132	110	66
20:4ω6	16	25	20	36	29	23
20:5ω3	128	171	214	310	267	147
20:4ω3	14	18	27	43	40	15
22:6ω3	372	485	528	679	608	437
22:5ω3	40	51	75	113	109	38
other	78	116	155	278	221	86
Total	1202	1535	2033	2980	2637	1311
Total SFA	351	426	579	831	706	349
Total MUFA	220	275	472	727	697	233
Total PUFA	631	833	983	1422	1234	730
Total ω3	570	747	879	1221	1076	655
Total ω6	45	66	76	144	116	61

Other:
i14:0,14:1,i15:0,a15:0,15:0,16PUFA,16:1ω9,16:2,16:1ω5,i17:0,a17:0,17:1,17:0,18:3ω6,18:3ω3,18:2, 18:1ω5,20:3ω6,20:2ω6,20:1ω11,20:1ω9,20:1ω7,20:0,21PUFA,22:5ω6,22:4ω6,22:3ω3,22:1ω11, 22:1ω9,22:1ω7,22:0,23PUFA,24PUFA,24:1,24:0, SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

Table 12.2. Lipid class and fatty acid composition of fresh and cold smoked Atlantic salmon - mid fillet

Lipid class	*Sample 1*		*Sample 2*		*Sample 3*	
	Fresh	Smoked	Fresh	Smoked	Fresh	Smoked
mg/100 g						
Triglyceride	173	3583	929	2200	357	540
Free fatty	4	13	3	7	8	22
Cholesterol	14	21	13	17	13	23
Polar oil	1065	1253	924	1025	1288	1447
Total	1256	4870	1868	3250	1666	2033
% Total lipid	1.3	4.9	1.9	3.2	1.7	2.0
% composition						
Triglyceride	13.8	73.6	49.7	67.7	21.4	26.5
Free fatty	0.3	0.3	0.2	0.2	0.5	1.1
Cholesterol	1.1	0.4	0.7	0.5	0.8	1.1
Polar oil	84.8	25.7	49.4	31.5	77.3	71.2

Fatty acid	*Sample 1*		*Sample 2*		*Sample 3*	
	Fresh	Smoked	Fresh	Smoked	Fresh	Smoked
mg/100 g						
14:0	14	186	56	132	24	33
16:1ω7	23	230	72	152	40	49
16:0	193	748	302	507	250	300
18:4ω3	8	82	27	60	15	19
18:2ω6	13	120	30	81	22	38
18:1ω9	77	616	193	386	129	183
18:1ω7	27	137	50	91	42	57
18:0	48	188	76	124	59	83
20:4ω6	13	44	16	33	18	22
20:5ω3	109	397	178	281	153	173
20:4ω3	11	65	22	42	18	22
22:6ω3	348	870	432	597	446	588
22:5ω3	35	164	67	110	54	60
other	52	428	124	299	81	122
Total	973	4275	1645	2895	1350	1748
Total SFA	268	1186	457	809	348	432
Total MUFA	151	1169	379	764	249	348
Total PUFA	554	1919	808	1321	753	969
Total ω3	512	1605	726	1108	687	870
Total ω6	34	209	60	147	52	78

Otherincludes12:0,i14:0,14:1,i15:0,a15:0,15:0,16PUFA,16:1ω9,16:2,16:1ω5,i17:0,a17:0,17:1,17:0, 18:3ω6,18:3ω3,18:2,18:1ω5,20:3ω6,20:2ω6,20:1ω11,20:1ω9,20:1ω7,20:0,21PUFA,22:5ω6,22:4ω6, 22:3ω3,22:1ω11,22:1ω9,22:1ω7,22:0,23PUFA,24PUFA,24:1,24:0, SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid

Table 12.3. Fatty acid composition (%) of Atlantic salmon – fresh, cold smoked, hot smoked, and Ishanabe products*. (mean±SD)

	Cold smoked		Hot smoked		Ishanabe	
	Fresh	Smoked	Fresh	Smoked	Fresh	Ishanabe
14:0	2.9 ±1.1	3.2 ±1.3	3.1 ±0.8	4.5 ±0.7	4.0 ±0.6	3.5 ±0.8
16:1ω7	3.8 ±1.1	3.9 ±1.3	4.0 ±0.8	5.8 ±0.6	5.2 ±0.6	4.6 ±0.6
16:0	18.8 ±1.1	18.0 ±0.8	21.3 ±0.9	17.4 ±0.7	17.8 ±0.6	18.0 ±0.8
18:4ω3	1.4 ±0.4	1.5 ±0.5	1.2 ±0.2	1.9 ±0.2	2.0 ±0.2	1.8 ±0.3
18:2ω6	1.8 ±0.4	2.3 ±0.5	1.8 ±0.2	2.8 ±0.2	2.4 ±0.3	2.4 ±0.3
18:1ω9	10.8 ±2.1	11.6 ±2.2	10.8 ±0.8	14.4 ±1.0	13.7 ±0.9	12.6 ±1.2
18:1ω7	2.9 ±0.2	3.0 ±0.3	3.2 ±0.3	3.3 ±0.2	3.1 ±0.1	3.1 ±0.1
18:0	4.6 ±0.3	4.6 ±0.3	5.0 ±0.4	4.0 ±0.3	4.5 ±0.2	4.5 ±0.2
20:4ω6	1.2 ±0.2	1.3 ±0.3	1.2 ±0.1	0.9 ±0.1	1.1 ±0.1	1.2 ±0.1
20:5ω3	10.8 ±0.4	10.3 ±0.8	9.9 ±0.7	9.5 ±0.7	10.5 ±0.5	10.6 ±0.6
20:4ω3	1.3 ±0.1	1.3 ±0.2	1.3 ±0.1	1.5 ±0.1	1.5 ±0.1	1.5 ±0.1
22:6ω3	29.2 ±4.8	27.0 ±6.4	26.6 ±2.6	20.0 ±2.5	21.9 ±2.0	23.4 ±3.0
22:5ω3	3.8 ±0.3	3.5 ±0.4	3.3 ±0.2	3.9 ±0.2	3.9 ±0.2	3.8 ±0.2
Other	6.9	8.5	7.3	10.1	8.4	9.1
Total SFA	27.6 ±1.2	27.1 ±1.3	30.7 ±0.8	27.4 ±0.9	27.7 ±0.5	27.4 ±0.7
Total MUFA	20.8 ±4.0	22.3 ±4.3	21.8 ±1.4	28.6 ±2.0	26.3 ±1.8	24.3 ±2.5
Total PUFA	51.6 ±4.2	50.6 ±5.1	47.6 ±1.5	44.0 ±2.7	45.9 ±1.7	48.3 ±2.6
Total ω3	46.5 ±4.6	43.8 ±6.0	42.3 ±1.9	36.8 ±2.9	39.9 ±2.0	41.1 ±3.2
Total ω6	3.8 ±0.3	4.7 ±0.3	3.7 ±0.3	4.7 ±0.1	4.3 ±0.2	4.6 ±0.3

* All treatments mean of 6 samples (3 x right shoulder, 3 x mid fillet)
Other includes 12:0,i14:0,14:1,i15:0,a15:0,15:0,16PUFA,16:1ω9,16:2,16:1ω5,i17:0,a17:0,17:1,17:0, 18:3ω6,18:3ω3,18:2,18:1ω5,20:3ω6,20:2ω6,20:1ω11,20:1ω9,20:1ω7,20:0,21PUFA,22:5ω6,22:4ω6, 22:3ω3,22:1ω11,22:1w9,22:1w7,22:0,23PUFA,24PUFA,24:1,24:0, SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid

Table 12.4. Lipid class and fatty acid composition of fresh and hot smoked Atlantic salmon - right shoulder

Lipid class	*Sample 1*		*Sample 2*		*Sample 3*	
	Fresh	Smoked	Fresh	Smoked	Fresh	Smoked
mg/100 g						
Triglyceride	769	1645	249	1702	308	2407
Free fatty	14	6	16	10	13	6
Cholesterol	16	5	21	27	18	17
Polar oil	1089	302	1225	1343	1303	1567
Total	1888	1958	1512	3084	1642	3997
% Total lipid	1.9	2.0	1.5	3.1	1.6	4.0
% composition						
Triglyceride	40.7	84.0	16.5	55.2	18.8	60.2
Free fatty	0.8	0.3	1.1	0.3	0.8	0.2
Cholesterol	0.8	0.3	1.4	0.9	1.1	0.4
Polar oil	57.7	15.4	81.1	43.6	79.3	39.2
Fatty acid	*Sample 1*		*Sample 2*		*Sample 3*	
	Fresh	Smoked	Fresh	Smoked	Fresh	Smoked
mg/100 g						
14:0	67	95	22	68	25	109
16:1w7	79	113	32	100	35	146
16:0	295	287	183	343	217	485
18:4ω3	24	37	10	30	12	52
18:2ω6	30	49	15	48	18	76
18:1ω9	173	256	92	262	105	386
18:1ω7	46	57	28	65	29	84
18:0	65	62	45	84	54	114
20:4ω6	16	13	11	19	14	28
20:5ω3	153	152	84	165	108	280
20:4ω3	21	27	11	27	12	40
22:6ω3	332	257	240	396	302	595
22:5ω3	52	65	29	70	32	104
other	107	177	59	180	76	277
Total	1460	1647	862	1856	1038	2774
Total SFA	446	471	260	521	308	746
Total MUFA	346	513	184	522	210	763
Total PUFA	668	663	417	814	520	1266
Total ω3	588	546	374	697	469	1085
Total ω6	55	79	33	84	39	131

Other includes12:0,i14:0,14:1,i15:0,a15:0,15:0,16PUFA,16:1ω9,16:2,16:1ω5,i17:0,a17:0, 17:1,17:0,18:3ω6,18:3ω3,18:2,18:1ω5,20:3ω6,20:2ω6,20:1ω11,20:1ω9,20:1ω7,20:0, 21PUFA,22:5ω6,22:4ω6,22:3ω3,22:1ω11,22:1ω9,22:1ω7,22:0,23PUFA,24PUFA,24:1, 24:0, SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid

Table 12.5. Lipid class and fatty acid composition of fresh and hot smoked Atlantic salmon - mid fillet

Lipid class	*Sample 1*		*Sample 2*		*Sample 3*	
	Fresh	Smoked	Fresh	Smoked	Fresh	Smoked
mg/100 g						
Triglyceride	240	1804	152	2612	292	2066
Free fatty	10	6	15	6	13	5
Cholesterol	15	20	19	14	14	17
Polar oil	727	1204	964	871	945	1061
Total	991	3033	1149	3503	1266	3150
% Total lipid	1.0	3.0	1.1	3.5	1.3	3.1
% composition						
Triglyceride	24.2	59.5	13.2	74.6	23.1	65.6
Free fatty	1.0	0.2	1.3	0.2	1.1	0.2
Cholesterol	1.5	0.6	1.6	0.4	1.1	0.5
Polar oil	73.3	39.7	83.9	24.9	74.7	33.7

Fatty acid	*Sample 1*		*Sample 2*		*Sample 3*	
	Fresh	Smoked	Fresh	Smoked	Fresh	Smoked
mg/100 g						
14:0	55	101	18	119	28	110
16:1ω7	70	124	23	156	33	135
16:0	327	382	159	423	189	420
18:4ω3	21	45	6	50	10	44
18:2ω6	33	57	11	77	15	68
18:1ω9	185	291	71	388	90	344
18:1ω7	52	71	26	90	25	75
18:0	76	84	39	101	43	96
20:4ω6	18	24	8	21	10	22
20:5ω3	163	240	61	223	83	225
20:4ω3	22	36	9	40	10	35
22:6ω3	383	518	201	478	231	471
22:5ω3	56	98	24	103	25	88
other	123	208	48	272	64	237
Total	1584	2280	704	2541	855	2369
Total SFA	479	598	223	679	270	659
Total MUFA	367	586	147	776	182	679
Total PUFA	738	1096	334	1086	403	1032
Total ω3	650	950	304	909	361	874
Total ω6	65	103	23	124	31	112

Other includes
12:0,i14:0,14:1,i15:0,a15:0,15:0,16PUFA,16:1ω9,16:2,16:1ω5,i17:0,a17:0,17:1,17:0, 18:3ω6,18:3ω3,18:2,18:1ω5,20:3ω6,20:2ω6,20:1ω11,20:1ω9,20:1ω7,20:0,21PUFA,22:5ω6, 22:4ω6,22:3ω3,22:1ω11,22:1ω9,22:1ω7,22:0,23PUFA,24PUFA,24:1,24:0, SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid

Table 12.6. Lipid class and fatty acid composition of Ishanabe Atlantic salmon - right shoulder

Lipid class	*Sample 1*		*Sample 2*		*Sample 3*	
	Fresh	Ishanabe	Fresh	Ishanabe	Fresh	Ishanabe
mg/100 g						
Triglyceride	2440	1619	1904	701	1349	437
Free fatty	4	9	5	4	6	15
Cholesterol	18	19	13	12	14	17
Polar oil	1272	1214	1088	977	1194	1120
Total	3733	2862	3009	1694	2563	1589
% Total lipid	3.7	2.9	3.0	1.7	2.6	1.6
% composition						
Triglyceride	65.4	56.6	63.3	41.4	52.7	27.5
Free fatty	0.1	0.3	0.2	0.3	0.2	1.0
Cholesterol	0.5	0.7	0.4	0.7	0.5	1.1
Polar oil	34.1	42.4	36.2	57.6	46.6	70.5

Fatty acid	*Sample 1*		*Sample 2*		*Sample 3*	
	Fresh	Ishanabe	Fresh	Ishanabe	Fresh	Ishanabe
mg/100 g						
14:0	134	101	94	42	69	35
16:1ω7	161	117	122	59	98	52
16:0	545	446	434	270	364	247
18:4ω3	61	48	45	23	37	19
18:2ω6	76	60	55	29	46	28
18:1ω9	443	320	320	165	263	151
18:1ω7	94	77	72	41	63	41
18:0	140	113	108	68	92	64
20:4ω6	33	30	27	18	25	20
20:5ω3	337	262	247	149	221	160
20:4ω3	45	35	37	20	31	20
22:6ω3	653	549	557	364	483	369
22:5ω3	119	89	95	52	82	53
other	273	239	192	114	166	103
Total	3114	2488	2404	1415	2040	1362
Total SFA	869	697	669	400	552	362
Total MUFA	842	623	607	313	510	289
Total PUFA	1403	1168	1127	702	978	711
Total ω3	1217	998	983	616	857	627
Total ω6	136	119	103	59	89	61

Other includes
12:0,i14:0,14:1,i15:0,a15:0,15:0,16PUFA,16:1ω9,16:2,16:1ω5,i17:0,a17:0,17:1,17:0,18:3ω6, 18:3ω3,18:2,18:1ω5,20:3ω6,20:2ω6,20:1ω11,20:1ω9,20:1ω7,20:0,21PUFA,22:5ω6,22:4ω6, 22:3ω3,22:1ω11,22:1ω9,22:1ω7,22:0,23PUFA,24PUFA,24:1,24:0, SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid

Table 12.7. Lipid class and fatty acid composition of Ishanabe Atlantic salmon - mid fillet

Lipid class	*Sample 1*		*Sample 2*		*Sample 3*	
	Fresh	Ishanabe	Fresh	Ishanabe	Fresh	Ishanabe
mg/100 g						
Triglyceride	1553	2418	2381	924	3643	1786
Free fatty	6	7	7	5	9	27
Cholesterol	18	18	12	14	19	23
Polar oil	1311	940	870	718	943	918
Total	2888	3383	3271	1662	4614	2754
% Total lipid	2.9	3.4	3.3	1.7	4.6	2.8
% composition						
Triglyceride	53.8	71.5	72.8	55.6	78.9	64.8
Free fatty	0.2	0.2	0.2	0.3	0.2	1.0
Cholesterol	0.6	0.5	0.4	0.9	0.4	0.8
Polar oil	45.4	27.8	26.6	43.2	20.4	33.3

Fatty acid	*Sample 1*		*Sample 2*		*Sample 3*	
	Fresh	Ishanabe	Fresh	Ishanabe	Fresh	Ishanabe
mg/100 g						
14:0	78	135	115	53	208	94
16:1ω7	102	159	156	74	260	127
16:0	433	503	515	318	711	421
18:4ω3	41	65	61	29	95	45
18:2ω6	51	83	64	38	123	63
18:1ω9	301	416	392	207	650	334
18:1ω7	77	93	90	53	131	81
18:0	112	127	128	73	177	107
20:4ω6	28	34	30	19	41	27
20:5ω3	260	295	279	177	420	253
20:4ω3	33	45	45	25	68	40
22:6ω3	552	570	598	427	798	514
22:5ω3	86	110	111	65	182	103
other	187	310	237	149	399	251
Total	2341	2945	2820	1707	4265	2462
Total SFA	655	812	801	469	1163	660
Total MUFA	577	809	751	398	1252	654
Total PUFA	1108	1324	1268	840	1850	1149
Total ω3	974	1104	1098	731	1567	973
Total ω6	100	152	118	75	198	118

Other includes
12:0,i14:0,14:1,i15:0,a15:0,15:0,16PUFA,16:1ω9,16:2,16:1ω5,i17:0,a17:0,17:1,17:0,
18:3ω6,18:3ω3,18:2,18:1ω5,20:3ω6,20:2ω6,20:1ω11,20:1ω9,20:1ω7,20:0,21PUFA,22:5ω6,
22:4ω6,22:3ω3,22:1ω11,22:1ω9,22:1ω7,22:0,23PUFA,24PUFA,24:1,24:0,SFA saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid

Table 12.8. Lipid class content (mg/100g) and composition (%) along an Atlantic salmon fillet.*

mg/100 g	Head			Middle			Tail	Whole fillet*
	1	2	3	4	5	6	7	
Triglyceride	640	5702	6880	6664	4998	3158	4416	5723 ±294
Free fatty acid	118	92	131	104	97	130	111	13 ±3
Cholesterol	32	32	40	48	44	35	48	17 ±7
Polar oil	1452	659	567	753	758	736	953	961 ±42
Total	2242	6485	7618	7568	5897	4059	5528	6713 ±261
%Total lipid	2.2	6.5	7.6	7.6	5.9	4.1	5.5	6.7 ±0.3
%								
Triglyceride	28.5	87.9	90.3	88.0	84.7	77.8	79.9	85.2 ±1.1
Free fatty acid	5.3	1.4	1.7	1.4	1.7	3.2	2.0	0.2 ±0.1
Cholesterol	1.4	0.5	0.5	0.6	0.7	0.9	0.9	0.2 ±0.1
Polar oil	64.8	10.2	7.4	9.9	12.9	18.1	17.2	14.3 ±1.1

* mean of three samples ± SD

Table 12.9. Fatty acid composition (%) along an Atlantic salmon fillet

	Head			Middle			Tail	Whole fillet*
	1	2	3	4	5	6	7	
14:0	3.3	5.5	5.2	5.5	5.6	4.9	5.3	4.7 ±0.1
16:1ω7	3.8	6.2	6.3	6.2	6.4	5.8	5.9	5.9 ±0.1
16:0	19.7	19.6	19.1	18.0	19.5	18.6	17.9	17.2 ±0.0
18:4ω3	1.5	2.4	2.6	3.0	2.2	2.2	2.4	3.1 ±0.0
18:2ω6	1.9	2.7	3.1	2.8	2.7	2.7	2.7	3.3 ±0.1
18:1ω9	10.4	14.4	15.5	14.5	14.6	14.2	14.1	15.4 ±0.1
18:1ω7	2.9	3.3	3.5	3.3	3.4	3.5	3.3	3.2 ±0.0
18:0	4.7	4.5	4.6	4.2	4.4	4.5	4.3	4.4 ±0.0
20:4ω6	1.3	0.8	0.8	0.8	0.8	0.9	0.9	0.8 ±0.1
20:5ω3	11.0	9.5	9.4	9.6	9.3	9.3	9.6	9.9 ±0.2
20:4ω3	1.2	1.4	1.5	1.5	1.5	1.4	1.5	1.3 ±0.0
22:6ω3	27.7	16.8	15.3	17.0	16.6	18.6	18.6	17.0 ±0.3
22:5ω3	3.4	3.6	3.5	3.8	3.5	3.7	3.8	3.2 ±0.1
Other	7.2	9.4	9.5	9.8	9.5	9.7	9.7	10.5
Total SFA	29.1	31.4	30.7	29.4	31.3	29.7	29.3	28.0 ±0.1
Total MUFA	20.3	27.8	29.4	28.2	28.4	27.9	27.6	28.7 ±0.2
Total PUFA	50.6	40.9	40.0	42.4	40.3	42.4	43.1	43.3 ±0.3
Total ω3	44.8	33.7	32.3	34.9	33.1	35.2	35.8	34.7 ±0.2
Total ω6	4.0	4.5	4.9	4.6	4.5	4.6	4.7	5.0 ±0.1

* mean of three samples ± SD

Other includes
12:0,i14:0,14:1,i15:0,a15:0,15:0,16PUFA,16:1ω9,16:2,16:1ω5,i17:0,a17:0,17:1,17:0,
18:3ω6,18:3ω3,18:2,18:1ω5,20:3ω6,20:2ω6,20:1ω11,20:1ω9,20:1ω7,20:0,21PUFA,22:5ω6,
22:4ω6,22:3ω3,22:1ω11,22:1ω9,22:1ω7,22:0,23PUFA,24PUFA,24:1,24:0, SFA, saturated fatty acid; MUFA monounsaturated fatty acid; PUFA, polyunsaturated fatty acid

Table 12.10. Fatty acid content (mg/100g) along an Atlantic salmon fillet

mg/100 g	Head			Middle			Tail	Whole fillet*
	1	2	3	4	5	6	7	
14:0	52	326	347	368	194	162	252	258 ±12
16:1ω7	59	364	421	413	223	192	281	327 ±17
16:0	305	1159	1273	1197	677	619	849	950 ±46
18:4ω3	23	139	172	199	77	73	111	172 ±7
18:2ω6	30	161	204	184	95	89	129	180 ±7
18:1ω9	161	850	1032	963	508	473	666	848 ±43
18:1ω7	46	195	234	221	117	116	157	179 ±9
18:0	72	264	305	277	152	150	203	241 ±12
20:4ω6	19	49	54	55	29	29	43	43 ±3
20:5ω3	170	559	626	635	325	308	454	546 ±20
20:4ω3	19	86	99	101	51	47	70	73 ±4
22:6ω3	430	993	1019	1133	576	618	879	940 ±49
22:5ω3	52	214	232	255	123	124	180	178 ±10
Other	111	554	633	649	330	321	460	582
Total	1549	5913	6650	6648	3478	3323	4735	5518 ±266
Total SFA	450	1854	2041	1954	1088	987	1387	1543 ±73
Total MUFA	315	1642	1953	1877	988	928	1308	1585 ±82
Total PUFA	784	2417	2657	2817	1402	1409	2041	2390 ±113
Total ω3	700	2023	2185	2362	1170	1188	1721	1931 ±96
Total ω6	63	266	323	308	158	152	223	277 ±7

* mean of three samples ± SD

Other includes
12:0,i14:0,14:1,i15:0,a15:0,15:0,16PUFA,16:1ω9,16:2,16:1ω5,i17:0,a17:0,17:1,17:0, 18:3ω6,18:3ω3,18:2,18:1ω5,20:3ω6,20:2ω6,20:1ω11,20:1ω9,20:1ω7,20:0,21PUFA,22:5ω6, 22:4ω6,22:3ω3,22:1ω11,22:1ω9,22:1ω7,22:0,23PUFA,24PUFA,24:1,24:0, SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid

Table 12.11. Lipid class composition (%) and content (mg/100g) of Atlantic salmon by-products.*

mg/100 g	Gut	Trim	Head	Frame	Skin	Salmon mince	Commercial burger
Wax ester	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0	52 ±7	27 ±17
Triglyceride	32712 ±3173	22739 ±225	18046 ±99	16750 ±782	13456 ±2305	18791 ±4628	9614 ±896
Free fatty acid	1201 ±190	35 ±24	25 ±9	34 ±4	25 ±0	19 ±32	43 ±9
Cholesterol	1417 ±173	23 ±3	48 ±6	47 ±11	67 ±13	219 ±329	38 ±14
Polar oil	750 ±31	624 ±29	756 ±20	727 ±198	561 ±219	6218 ±9311	123 ±15
Total	36157 ±3571	23422 ±223	18901 ±45	17596 ±554	14236 ±2052	25298 ±5038	9844 ±902
%Total lipid	36.2 ±3.6	23.4 ±0.2	18.9 ±0.0	17.6 ±0.6	14.2 ±2.1	25.3 ±5.0	9.8 ±0.9
%							
Wax ester	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.2 ±0.1	0.3 ±0.2
Triglyceride	90.5 ±0.2	97.1 ±0.0	95.5 ±0.3	95.2 ±1.4	94.3 ±2.6	78.3 ±30.4	97.6 ±0.4
Free fatty acid	3.3 ±0.2	0.2 ±0.1	0.1 ±0.0	0.2 ±0.0	0.2 ±0.0	0.1 ±0.1	0.4 ±0.1
Cholesterol	3.9 ±0.1	0.1 ±0.0	0.3 ±0.0	0.3 ±0.1	0.5 ±0.2	0.7 ±1.0	0.4 ±0.1
Polar oil	2.1 ±0.1	2.7 ±0.1	4.0 ±0.1	4.2 ±1.3	4.1 ±2.1	20.7 ±29.3	1.2 ±0.0

* mean of three samples ± SD

Table 12.12. Fatty acid composition (%) of Atlantic salmon by-products.*

	Gut	Trim	Head	Frame	Skin	Salmon mince	Commercial burger
14:0	4.7 ±0.0	4.6 ±0.0	4.8 ±0.2	4.4 ±0.1	4.8 ±0.0	2.8 ±0.9	2.6 ±0.1
16:1ω7	6.4 ±0.1	6.1 ±0.1	5.9 ±0.1	5.9 ±0.0	6.0 ±0.0	5.6 ±1.0	3.5 ±0.2
16:0	15.3 ±0.1	16.1 ±0.0	16.7 ±0.1	16.2 ±0.0	16.2 ±0.0	14.0 ±1.6	11.0 ±0.5
18:4ω3	3.4 ±0.0	3.3 ±0.0	3.0 ±0.0	3.2 ±0.0	3.1 ±0.0	0.8 ±0.1	1.6 ±0.1
18:2ω6	3.9 ±0.0	3.5 ±0.0	3.6 ±0.1	3.6 ±0.0	3.6 ±0.0	2.9 ±0.1	12.8 ±1.6
18:1ω9	16.9 ±0.1	16.3 ±0.1	16.6 ±0.2	16.3 ±0.1	16.4 ±0.0	17.4 ±0.4	35.9 ±0.7
18:1ω7	3.3 ±0.1	3.3 ±0.0	3.2 ±0.0	3.3 ±0.0	3.3 ±0.0	3.5 ±0.3	3.3 ±0.1
18:0	4.2 ±0.1	4.1 ±0.0	4.4 ±0.0	4.2 ±0.1	4.2 ±0.0	5.2 ±1.7	3.3 ±0.1
20:4ω6	0.9 ±0.0	0.9 ±0.0	0.9 ±0.1	1.0 ±0.0	1.1 ±0.0	1.5 ±0.6	0.4 ±0.0
20:5ω3	9.2 ±0.1	10.2 ±0.0	9.7 ±0.4	10.1 ±0.1	10.0 ±0.1	10.6 ±0.3	5.1 ±0.3
20:4ω3	1.4 ±0.0	1.4 ±0.0	1.2 ±0.0	1.3 ±0.0	1.3 ±0.0	1.7 ±0.1	0.8 ±0.1
22:6ω3	14.7 ±0.1	15.4 ±0.0	14.9 ±0.3	15.5 ±0.0	14.7 ±0.1	18.8 ±2.4	8.3 ±0.5
22:5ω3	3.3 ±0.1	3.4 ±0.0	3.0 ±0.1	3.3 ±0.0	3.2 ±0.0	5.3 ±0.3	2.1 ±0.1
Other	12.4	11.5	11.8	11.9	12.0	9.9	9.3
Total SFA	26.0 ±0.1	26.5 ±0.0	27.8 ±0.4	26.5 ±0.0	27.0 ±0.0	21.1 ±3.5	16.8 ±0.6
Total MUFA	31.4 ±0.1	30.0 ±0.1	30.7 ±0.4	30.2 ±0.0	30.5 ±0.0	35.5 ±0.2	47.4 ±0.6
Total PUFA	42.6 ±0.0	43.4 ±0.2	41.5 ±0.8	43.3 ±0.0	42.5 ±0.1	48.6 ±2.8	37.6 ±0.2
Total ω3	32.2 ±0.1	33.7 ±0.0	32.0 ±0.8	33.4 ±0.1	32.5 ±0.1	39.5 ±0.8	31.5 ±0.1
Total ω6	5.9 ±0.1	5.4 ±0.0	5.4 ±0.1	5.6 ±0.0	5.7 ±0.0	9.1 ±2.0	6.1 ±0.3

* mean of three samples ± SD

Other includes 12:0,i14:0,14:1,i15:0,a15:0,15:0,16PUFA,16:1ω9,16:2,16:1ω5,i17:0,a17:0,17:1,17:0,18:3ω6,18:3ω3,18:2,18:1ω5, 20:3ω6,20:2ω6,20:1ω11,20:1ω9,20:1ω7,20:0,21PUFA,22:5ω6,22:4ω6,22:3ω3,22:1ω11,22:1ω9,22:1ω7,22:0,23PUFA,24PUFA,24:1,24:0, SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid

Table 12.13. Fatty acid content (mg/100g) of Atlantic salmon by-products.*

mg/100 g	Gut	Trim	Head	Frame	Skin	Salmon mince	Commercial burger
14:0	1459 ±145	939 ±21	791 ±42	669 ±32	587 ±91	442 ±45	259 ±6
16:1ω7	1979 ±206	1244 ±22	967 ±28	896 ±37	730 ±112	918 ±86	350 ±4
16:0	4745 ±400	3269 ±91	2734 ±56	2470 ±76	1969 ±297	2314 ±374	1093 ±24
18:4ω3	1063 ±85	669 ±21	498 ±0	486 ±19	382 ±58	126 ±24	156 ±4
18:2ω6	1217 ±109	719 ±22	590 ±20	546 ±21	433 ±65	491 ±129	1288 ±234
18:1ω9	5231 ±465	3304 ±73	2717 ±63	2492 ±63	1991 ±291	2960 ±932	3591 ±270
18:1ω7	1008 ±74	665 ±24	527 ±10	498 ±10	405 ±61	606 ±230	331 ±25
18:0	1294 ±99	837 ±23	716 ±11	646 ±14	512 ±71	939 ±586	325 ±7
20:4ω6	295 ±39	178 ±6	147 ±9	149 ±5	128 ±18	271 ±195	40 ±2
20:5ω3	2863 ±220	2082 ±53	1592 ±37	1540 ±41	1211 ±172	1783 ±466	508 ±5
20:4ω3	433 ±49	278 ±7	203 ±1	202 ±6	161 ±24	289 ±97	79 ±1
22:6ω3	4564 ±385	3126 ±90	2428 ±19	2362 ±78	1789 ±258	3253 ±1387	827 ±8
22:5ω3	1027 ±114	684 ±18	494 ±6	498 ±18	390 ±58	907 ±326	209 ±2
Other	3864	2337	1931	1824	1458	1603	857
Total	31042 ±2883	20331 ±549	16335 ±222	15279 ±512	12147 ±1808	16901 ±4874	9983 ±563
Total SFA	8075 ±717	5397 ±144	4537 ±120	4052 ±138	3284 ±493	3917 ±931	1814 ±39
Total MUFA	9737 ±937	6103 ±136	5012 ±139	4612 ±149	3700 ±554	5179 ±1359	4809 ±321
Total PUFA	13230 ±1229	8831 ±269	6786 ±37	6616 ±225	5163 ±761	7805 ±2585	3361 ±216
Total ω3	10266 ±914	7038 ±201	5368 ±61	5242 ±167	4049 ±590	6357 ±2299	1779 ±18
Total ω6	1845 ±194	1090 ±33	877 ±3	849 ±35	689 ±106	955 ±385	1390 ±231

* mean of three samples ± SD

Other includes
12:0,i14:0,14:1,i15:0,a15:0,15:0,16PUFA,16:1ω9,16:2,16:1ω5,i17:0,a17:0,17:1,17:0,18:3ω6,18:3ω3,18:2,18:1ω5,20:3ω6,20:2ω6,20:1ω11,2
0:1ω9,20:1ω7,20:0,21PUFA,22:5ω6,22:4ω6,22:3ω3,22:1ω11,22:1ω9,22:1ω7,22:0,23PUFA,24PUFA,24:1,24:0, SFA, saturated fatty acid;
MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid

Table 12.14. Lipid class composition (%) and content (mg/100g) of Atlantic salmon caviar.*

	Fresh Caviar		Preserved Caviar
mg/100 g	Unsalted	Salted	Salted and Stored
Wax ester	85 ±22	31 ±11	28 ±9
Triglyceride	4374 ±137	6456 ±1606	5208 ±1717
Free fatty acid	25 ±15	61 ±57	34 ±22
Cholesterol	153 ±23	249 ±83	161 ±119
Polar oil	5314 ±467	7894 ±1833	5191 ±4485
Total	9950 ±342	14690 ±3552	10623 ±3195
%Total lipid	10.0 ±0.3	14.7 ±3.6	10.6 ±3.2
%			
Wax ester	0.8 ±0.2	0.2 ±0.0	0.3 ±0.1
Triglyceride	44.0 ±2.7	43.9 ±0.4	54.7 ±33.1
Free fatty acid	0.3 ±0.2	0.4 ±0.3	0.4 ±0.3
Cholesterol	1.5 ±0.3	1.7 ±0.3	1.4 ±0.8
Polar oil	53.3 ±3.0	53.8 ±0.8	43.2 ±32.8

* mean of three samples ± SD

Table 12.15. Fatty acid composition (%) and content (mg/100g) of Atlantic salmon caviar.*

	Fresh Caviar		Preserved Caviar
%	Unsalted	Salted	Salted and Stored
14:0	2.3 ±0.1	2.4 ±0.1	2.4 ±0.7
16:1ω7	4.6 ±0.1	5.1 ±0.1	4.7 ±1.1
16:0	13.4 ±0.1	13.1 ±0.2	13.5 ±1.5
18:4ω3	1.1 ±0.0	1.3 ±0.0	0.7 ±0.1
18:2ω6	2.2 ±0.1	2.4 ±0.2	2.9 ±0.3
18:1ω9	12.4 ±0.2	13.3 ±0.1	16.8 ±0.6
18:1ω7	3.4 ±0.1	3.5 ±0.0	3.7 ±0.2
18:0	6.7 ±0.1	7.6 ±0.2	6.4 ±1.7
20:4ω6	2.4 ±0.1	2.2 ±0.0	2.0 ±0.7
20:5ω3	14.1 ±0.1	13.4 ±0.2	10.4 ±0.4
20:4ω3	1.8 ±0.0	2.0 ±0.0	1.6 ±0.0
22:6ω3	22.8 ±0.4	20.7 ±0.1	21.0 ±3.1
22:5ω3	6.5 ±0.1	6.6 ±0.1	5.3 ±0.1
Other	6.4	6.2	8.8
Total SFA	18.0 ±0.3	17.7 ±0.1	19.5 ±3.0
Total MUFA	29.9 ±0.4	32.0 ±0.2	33.8 ±1.5
Total PUFA	58.0 ±1.4	56.6 ±1.2	54.6 ±2.3
Total ω3	46.5 ±1.3	44.2 ±1.1	44.0 ±1.2
Total ω6	11.5 ±0.2	12.4 ±0.2	10.6 ±2.2
mg/100 g			
14:0	183 ±8	199 ±7	149 ±18
16:1ω7	359 ±14	436 ±21	300 ±25
16:0	1054 ±73	1115 ±95	871 ±105
18:4ω3	86 ±5	107 ±11	47 ±8
18:2ω6	170 ±4	202 ±7	186 ±30
18:1ω9	979 ±62	1131 ±86	1100 ±229
18:1ω7	265 ±14	298 ±21	245 ±66
18:0	530 ±39	646 ±66	434 ±181
20:4ω6	190 ±15	188 ±15	135 ±67
20:5ω3	1110 ±72	1138 ±97	679 ±129
20:4ω3	145 ±9	173 ±12	103 ±23
22:6ω3	1797 ±147	1757 ±141	1411 ±477

Table 12.15 (continued)

22:5ω3	511 ±38	564 ±43	352 ±84
Other	501	525	566
Total	7882 ±528	8479 ±644	6576 ±1431
Total SFA	1851 ±129	2051 ±173	1540 ±283
Total MUFA	1778 ±109	2039 ±139	1882 ±357
Total PUFA	4253 ±293	4389 ±334	3155 ±800
Total ω3	3649 ±269	3739 ±303	2592 ±717
Total ω6	433 ±20	461 ±20	401 ±116

* mean of three samples ± SD
Other includes12:0,i14:0,14:1,i15:0,a15:0,15:0,16PUFA,16:1ω9,16:2,16:1ω5,i17:0,a17:0,17:1, 17:0,18:3ω6,18:3ω3,18:2,18:1ω5,20:3ω6,20:2ω6,20:1ω11,20:1ω9,20:1ω7,20:0,21PUFA,22:5ω6, 22:4ω6,22:3ω3,22:1ω11,22:1ω9,22:1ω7,22:0,23PUFA,24PUFA,24:1,24:0, SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid

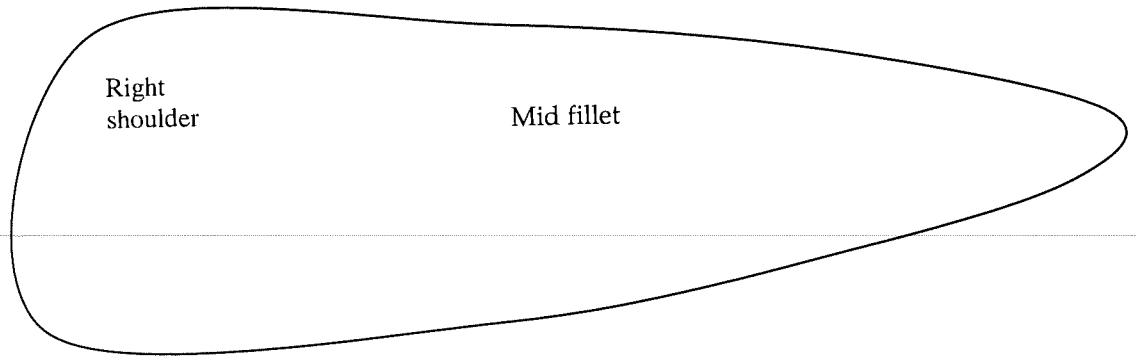


Figure12.1 – Sampling locations for cold smoking, hot smoking and salting (Ishanabe) treatments

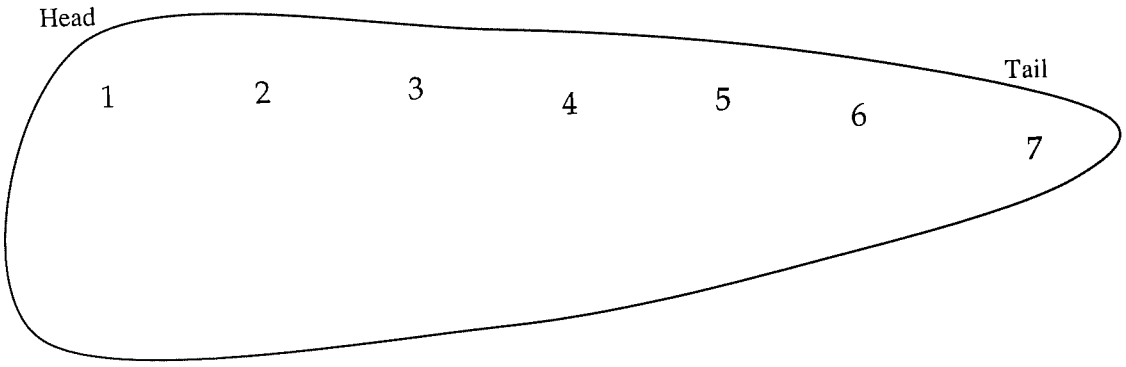


Figure 12.2. Sampling locations along the fillet

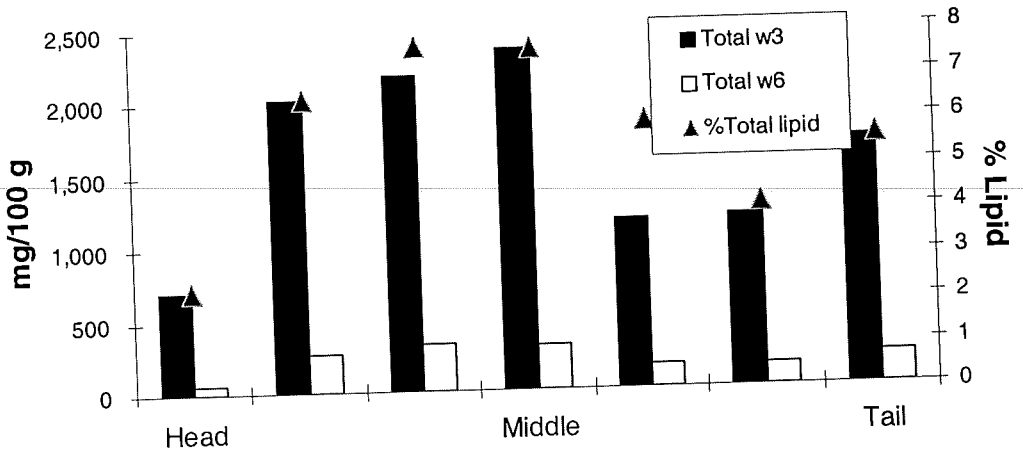


Figure 12.3. Content of omega-3 and omega-6 LC-PUFA, and total lipid (oil) along an Atlantic salmon fillet

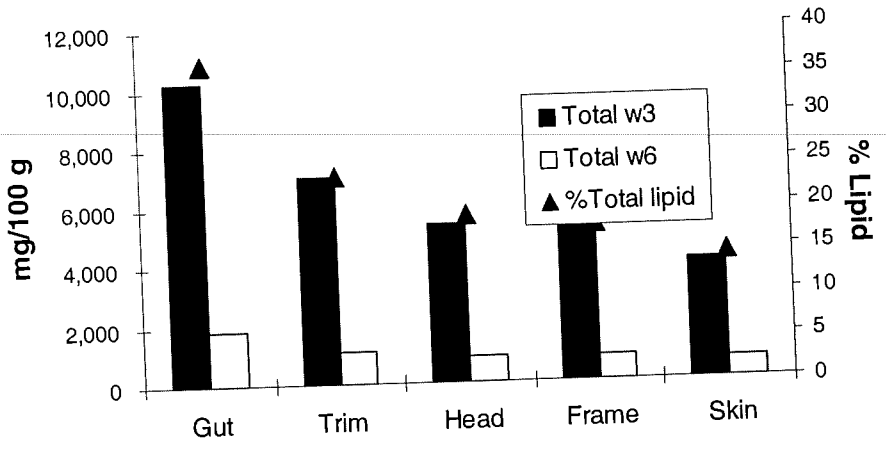


Figure 12.4. Content of omega-3 and omega-6 LC-PUFA, and total lipid (oil) in Atlantic salmon by-product

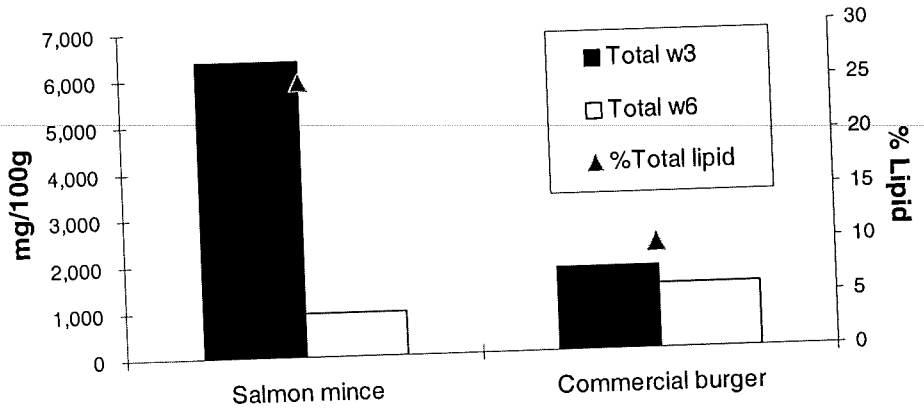


Figure 12.5. Content of omega-3 and omega-6 LC-PUFA, and total lipid (oil) in Atlantic salmon mince and commercial fish burger

13. The Oil Content and Composition of Cultured Barramundi

Results in this chapter were provided as a report to Bluewater Barramundi.
Mooney, B., Elliott, N., and Nichols, P. (2001) The oil content and composition of cultured barramundi. Report 2001-BWB1.

13.1 Summary

The oil content and composition of cultured barramundi supplied by Bluewater Barramundi, Queensland was detemined. Emphasis was placed on the levels of the beneficial long-chain (C_{20} and C_{22}) omega-3 polyunsaturated fatty acids (LC-PUFA), in particular eicosapentaenoic acid [EPA, 20:5(n-3)] and docosahexaenoic acid [DHA, 22:6(n-3)].

The fillets of cultured barramundi contained an oil content of 10% (wet weight basis). In comparison, the average oil content of Australian wild-caught fish is around 1% (range 0.3-25%), with wild-caught barramundi ranging from 0.4% (saltwater) to 0.9% (freshwater). The omega-3 LC-PUFA, predominantly DHA and EPA, accounted for 22% of the total fatty acids in cultured barramundi. DHA was the dominant PUFA at 10%. On an absolute basis, the omega-3 LC-PUFA content was 1966 mg/100 g, comparable to other species regarded as good sources of these beneficial oils. Based on the results of this study, barramundi cultured on a suitable diet can be an excellent source of the beneficial omega-3 LC PUFA.

13.2 Introduction

There is increasing nutritional interest in the long-chain (C_{20} and C_{22}) omega-3 polyunsaturated fatty acids (termed LC-PUFA). In particular, eicosapentaenoic acid [EPA, 20:5(n-3)] and docosahexaenoic acid [DHA, 22:6(n-3)] in seafood and marine oil products receive special attention. This is the result of well-documented nutritional benefits of these unique LC-PUFA. They help against coronary heart disease, high blood pressure, rheumatoid arthritis, and may also be beneficial against other disorders, including some forms of cancer, depression and other neural illnesses. Most Australian seafood contains elevated levels of the long-chain omega-3 oils, including the beneficial DHA and EPA (Table 13). The oil composition of 200 species of Australian seafood is available in the Guide *Seafood the Good Food* and in an accompanying FRDC report (Nichols *et al.* 1998a&b).

Whilst considerable data now exists on the oil composition of wild-caught Australian seafood, less information is available for cultured species. In this study, we report on the oil content and composition of cultured barramundi supplied by Bluewater Barramundi, Queensland

This study was performed as part of a Fisheries Research and Development Corporation (FRDC) supported investigation (1999/331) of the oil composition of Australian Seafood.

13.3 Materials and Methods

13.4 Samples

Four specimens of farmed barramundi were provided by Bluewater Barramundi, Queensland. The samples were fillet portions with the skin removed, and had been sealed in plastic bags, and transported frozen by overnight air-freight to Hobart.

13.5 Oil analyses

Oil (lipid) analyses were conducted using methods developed during FRDC projects 1995/122 and 1999/331. Full details are provided in reports prepared for FRDC (Nichols *et al.* 1998a&b), with the methods also published in the peer-reviewed literature (e.g. Bakes *et al.* 1995, Nichols *et al.* 1998c). Briefly, samples were extracted using a single phase Bligh and Dyer (1959) procedure. Oil yield was determined gravimetrically. An aliquot of the oil was analysed by TLC-FID to determine lipid class composition. Fatty acid profiles were obtained by capillary GC and GC-MS analysis following transmethylation of an aliquot of the extracted oil.

13.6 Results and Discussion

Cultured barrramundi supplied by Bluewater Barramundi contained 10% oil (wet weight basis), with triglyceride as the main oil class (97%; Table 13.1, Fig. 13.1).

The main fatty acids in cultured barramundi in decreasing order of abundance were: 18:1(n-9), 16:0, DHA, 16:1(n-7), EPA, 14:0, 18:2(n-6) and 18:0 (Table 13.2). These eight components accounted for 77% of the total fatty acids.

The omega-3 LC-PUFA, predominantly DHA and EPA, accounted for 22% of the total fatty acids, with total PUFA at 29%. DHA was the dominant PUFA at 10% (Table 13.2). The relative (percent) level omega-3 LC-PUFA in cultured barramundi was similar to wild-caught freshwater barramundi (26%), but lower than in wild-caught saltwater barramundi (43%). The ratio (n-3) PUFA / (n-6) PUFA was higher (3.0) in cultured fish compared to the wild fish (0.8-1.9).

On an absolute basis, the omega-3 LC-PUFA content of cultured barramundi was 1966 mg/100 g (Table13.2, Fig. 13.2). This value is markely higher than the average for wild-caught Australian fish (210 mg/100 g; Nichols *et al.* 1998a&b); levels in wild fish are around five to tenfold or more greater than occurs in other food groups (Table 13.3). In comparison to wild-caught fish, cultured fish fed marine oil based diets generally contain higher levels of the beneficial oils (Table 13.3).

High levels of oil and omega-3 LC-PUFA are often found in lower-value cuts and body parts, with highest values occurring in the belly-flap/viscera, followed by the frame and head. It may be possible to use some of these materials in the preparation of value-added products such as burger mince and soups.

In companion studies with other fish species, we have also examined the effect of various forms of cooking on oil and omega-3 LC-PUFA content (Chapter 17). The oil content of the

fish varied depending on the method of cooking. With microwave or steam cooking, oil and omega-3 LC-PUFA content and composition were generally similar to fresh fish. Oil content of fish increased when fried or grilled. The higher oil content observed is consistent with uptake of cooking oil by the fillet, with the oil composition also influenced by the type of oil used - omega-6 PUFA and oleic acid increased when peanut and cottonseed oil were used respectively. Importantly, the content of the omega-3 LC-PUFA, mainly DHA and EPA, was largely not affected by any of the forms of cooking.

Under the current feeding practices cultured barramundi is an excellent source of the beneficial omega-3 LC-PUFA. Value-adding treatments such as smoking and cooking do not diminish the omega-3 LC-PUFA content. Processing by-product ("waste") also offers unique opportunities for value-adding. Should fish meal and fish oil in current diets be replaced with other materials, the current high levels of omega-3 LC-PUFA may decrease, as may the product and nutritional value.

13.7 Conclusion

The current study represents, to our knowledge, one of the first detailed comparisons on the oil content and composition of cultured barramundi.

High oil and LC-omega-3 PUFA content has been found for fillets of cultured barramundi. The levels of LC-omega-3 PUFA are comparable to results for other cultured species, including Tasmanian Atlantic salmon and jade perch.

Products such as soups and burgers can be derived from seafood processing waste, and such by-products can provide good levels of omega-3 LC-PUFA. Such value-adding may be possible with barramundi by-products.

Scope to further increase the level of beneficial oils in cultured barramundi through manipulation of diet may also exist.

Table 13.1. Lipid content and composition of cultured barramundi.

Lipid class	Percentage composition
Triglyceride	97.4±0.7
Free fatty acid	0.2±0.0
Cholesterol	0.2±0.0
Phospholipid	2.2±0.6
Oil content (wet weight basis)	10.0±1.2

Table 13.2. Fatty acid composition and content of cultured barramundi.

Fatty acid	Percentage composition
14:0	5.9±0.1
i15:0	0.2±0.0
15:0	0.6±0.0
C16PUFA	1.5±0.1
16:1(n-7)c	6.5±0.2
16:0	19.0±0.4
17:1(n-8)/a17:0	0.5±0.0
18:4(n-3)	1.9±0.1
18:2(n-6)	5.5±0.1
18:1(n-9)c	19.6±0.6
18:1(n-7)c	2.9±0.0
18:0	4.4±0.1
20:4(n-6) AA	0.6±0.0
20:5(n-3) EPA	6.2±0.3
20:4(n-3)	0.8±0.0
20:1(n-9)c	3.5±0.4
22:6(n-3) DHA	10.2±0.4
22:5(n-3)	2.5±0.2
22:1(n-11)	1.6±0.1
22:1(n-9)	0.5±0.1
24:1	0.5±0.1
Other	5.0±0.1
Total	100.0
Percent	
Sum SAT	31.1±0.4
Sum MUFA	37.0±0.4
Sum PUFA	30.3±0.1
Sum n-3	21.6±0.1
Sum n-6	7.2±0.1
mg/100g	
Sum SAT	2835±352
Sum MUFA	3368±383
Sum PUFA	2755±317
Sum n-3	1966±222
Sum n-6	651±74

Table 13.3. Summary of average content of LC omega-3 PUFA in Australian seafood and comparison to other food groups.

Food group	LC omega-3 PUFA mg/100 g
Australian seafood (wild)	
Fish	**210**
Oysters	**150**
Prawns	**120**
Lobster	**105**
Farmed Australian fish	
Atlantic salmon	1930
Striped perch	2480
Silver perch	790
Barramundi	1966
Other food groups	
Turkey	35
Beef	22
Chicken	19
Lamb	18
Pork	0
Veal	0

Data for non-seafood items from Cashel *et al.* (1989), Mann *et al.* (1995), Sinclair *et al.* (1983).

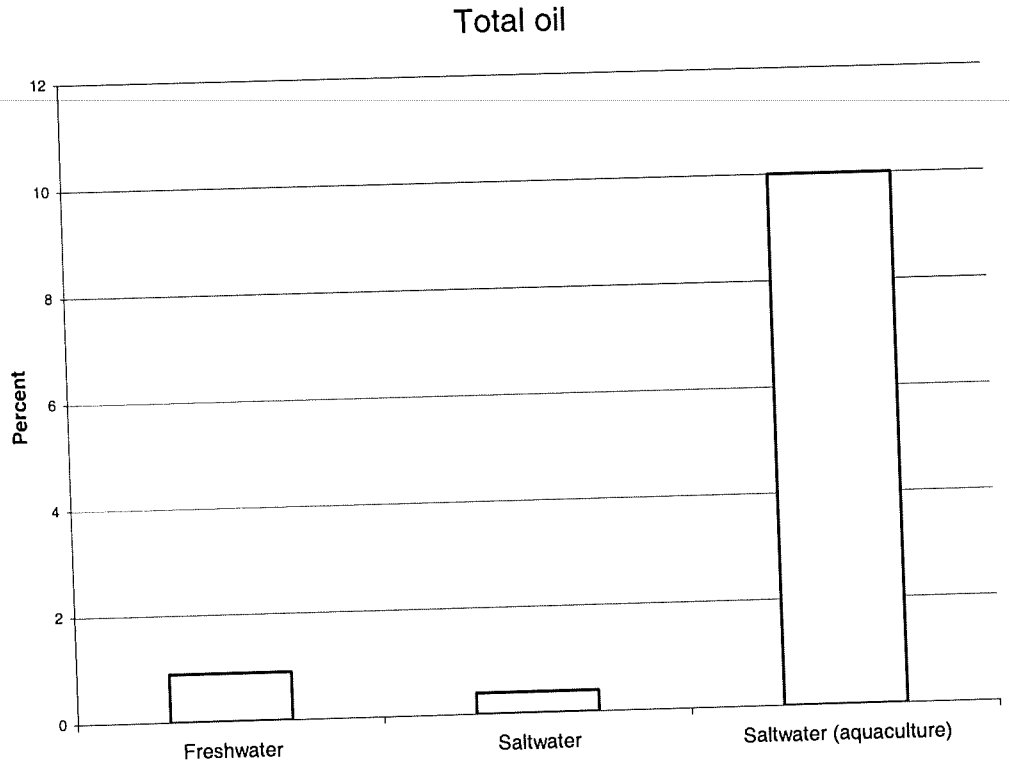


Figure 13.1. Content of oil in wild-caught (freshwater and saltwater) and cultured barramundi.

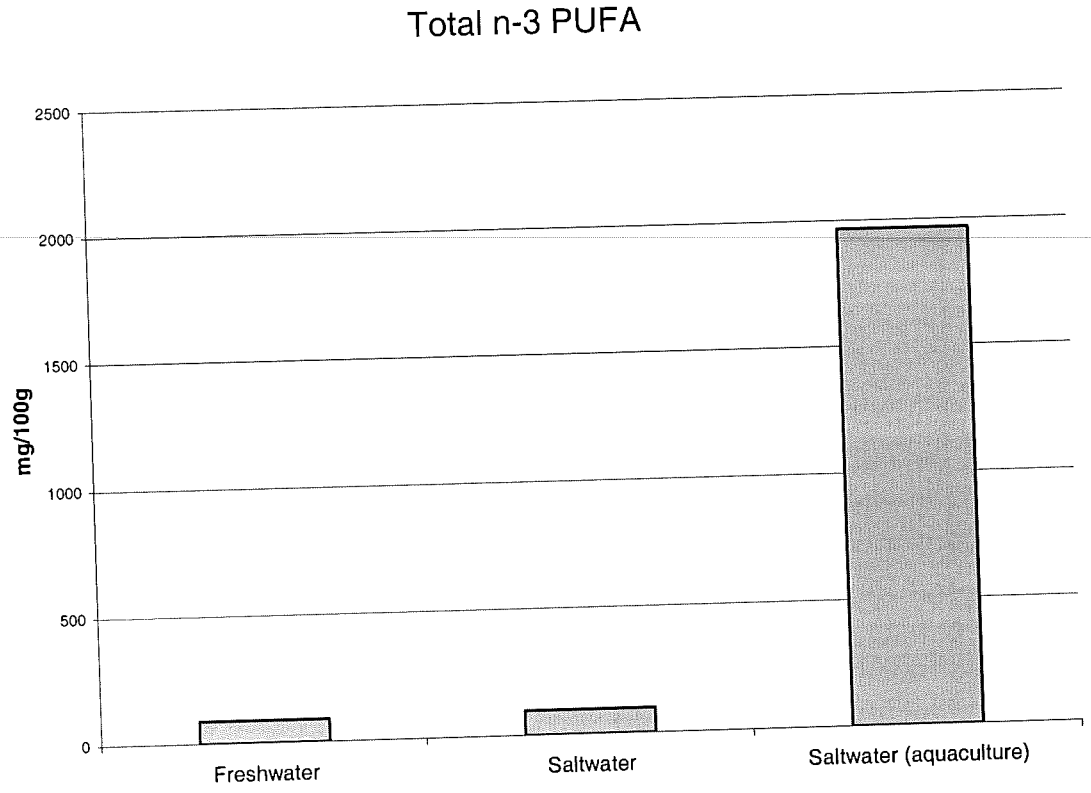


Figure 13.2. Content of omega-3 LC-PUFA in wild-caught (freshwater and seawater) and cultured barramundi.

14. Oil Content and Composition of Fillet Samples of Farmed Striped Trumpeter

Results in this chapter were provided as a report to the Tasmanian Aquaculture and Fisheries Institute and Tassal.
Mooney, B., Elliott, N. and Nichols, P. (2001) Oil content and composition of fillet samples of farmed striped trumpeter. Internal Report 2001-CMR1.

14.1 Introduction

The oil content and composition, and fatty acid composition were determined for fillet samples of farmed striped trumpeter (*Latris lineata*). Samples consisted of frozen flesh, received from Tassal in November 2000.

Analytical methods used were thin layer chromatography – flame ionisation detection (TLC-FID) and gas chromatography (GC)/GC-mass spectrometry (GC-MS). Sample work-up and analytical methods were as described in Nichols *et al.* (1994, 1998a).

This study was performed as part of an FRDC-funded investigation (1999/331) of the oil composition of Australian seafood.

Two fillets were analysed:

- A – from a wild-caught fish held in culture for 18 months on a salmon feed, then 1-2 months on a squid/mackerel diet, prior to slaughter
- A1 – from a wild caught fish held for 1-2 months and fed on the squid/mackerel diet, prior to slaughter

White and dark flesh was subsampled from the fillets for analysis. The A fillet contained obvious fat "seams" or deposits between the myomeres of the fillet.

14.2 Results and Discussion

Oil content and class composition

Total oil (lipid) content of the striped trumpeter samples ranged from 1.5% to 46% (wet weight basis), with the highest oil content in the dark tissue from the A fillet (Table 14.1).

Triglyceride was the major lipid class in all samples (48-99%), with lower levels of phospholipid (1-44%) and free fatty acid (0.1-6%). High oil content correlated well with high triglyceride levels, which is in agreement with previous findings of FRDC-funded research on the oil composition of Australian seafood (Nichols *et al.* 1998a&b).

Fatty acid composition

The major fatty acids in the striped trumpeter samples were 18:1ω9 (15-25%), DHA (10-25%), 16:0 (15-16%) and EPA (5-8%) (Table 14.2). These 4 components accounted for 53-62% of the total fatty acids in all samples.

Monounsaturated fatty acids (MUFA) (40-43%) were dominant over polyunsaturated fatty acids (PUFA) (30-33%) and saturated fatty acids (SAT) (27%) in all samples except the white flesh of the A1 fillet. In this sample, the PUFA was highest at 42%, with 31% MUFA and 27% SFA. The white flesh of the A1 also contained the highest relative levels of omega-3 PUFA. The highest absolute quantity of omega-3 PUFA, however, was recorded in the dark tissue of the A fillet, with very high quantities also in the white tissue of this fillet and in the dark tissue of the A1 fillet.

The results for the white flesh of the A1 fillet are the most similar to the fatty acid profile of wild caught striped trumpeter previously reported (Table 14.2) (Nichols *et al.* 1998b). The wild caught specimens, however, had lower levels of MUFA (20%) and higher levels of PUFA (55%), in particular DHA at 42%. The ratio of (omega-3)/(omega-6) was also higher in the wild specimens.

There is ever increasing interest in the long chain polyunsaturated fatty acids found in seafood and marine oil products. The nutritional benefits for humans is well documented (Nichols *et al.* 1998a & b), and most Australian seafood contain elevated levels of long chain omega-3 fatty acids, in particular DHA and EPA.

The observed omega-3 PUFA content value in the dark flesh of fillet A striped trumpeter (10200mg/100g) is extremely high. This value represents to our knowledge one of the highest reported levels to date of omega-3 PUFA in Australian seafood (mean 210mg/100g, range 30 to 1100mg/100g; Nichols *et al.* 1998a & b; Figure 14.1). Relative to commercially available fish oil supplements, one 100g serving of the oil-rich striped trumpeter would provide the equivalent of thirty capsules of 1000mg of fish oil containing omega-3 PUFA. The possible availability of such a high dose of omega-3 PUFA in a meal may be of considerable interest to medical and health practitioners seeking to treat a range of disorders with omega-3 PUFA. The very high oil level (>30%), however, may not be entirely acceptable for all forms of processing or from a palatability and taste aspect.

The oil composition results for striped trumpeter both on culture diets and wild specimens, show this species as an excellent source of omega-3 fatty acids. These results also clearly show that the oil composition of cultured species can be suitably manipulated via formulated diets.

Table 14.1. Lipid class composition and lipid content (%) of striped trumpeter samples.

Lipid class	Fillet A		Fillet A1	
	White flesh	Dark flesh	White flesh	Dark flesh
Triglyceride	98.7	98.4	47.9	89.2
Free fatty acid	0.1	0.4	6.2	3.5
Cholesterol	0.2	0.2	1.6	0.6
Phospholipid	1.0	1.0	44.3	6.7
Total lipid (%)	**31.1**	**46.2**	**1.5**	**8.8**

Table 14.2. Percentage composition of main individual fatty acids and fatty acid classes in striped trumpeter samples, and equivalent amounts of omega-3 fatty acids (mg wet weight) in 100g of tissue.

Fatty acid	**Fillet A**		**Fillet A1**		**Wild specimen**
	White flesh	Dark flesh	White flesh	Dark flesh	(from Nichols *et al.* 1998b)
16:0	15.1	15.3	16.2	14.8	16.5
18:1ω9	25.3	24.8	14.7	17.1	8.3
20:5ω3 EPA	8.4	7.7	5.9	5.3	3.6
22:6ω3 DHA	9.7	10.1	25.0	16.2	41.8
Total SAT	26.8	27.1	27.2	26.8	24.5
Total MUFA	42.4	42.5	30.6	40.4	20.2
Total PUFA	30.8	30.4	42.2	32.8	54.8
Total ω3	23.6	23.3	35.8	27.2	50.0
Total ω6	5.2	5.4	5.4	4.7	3.8
ω3/ω6	4.5	4.3	6.6	5.8	13.2
ω3 (mg/100g)	7200	10200	450	2250	358

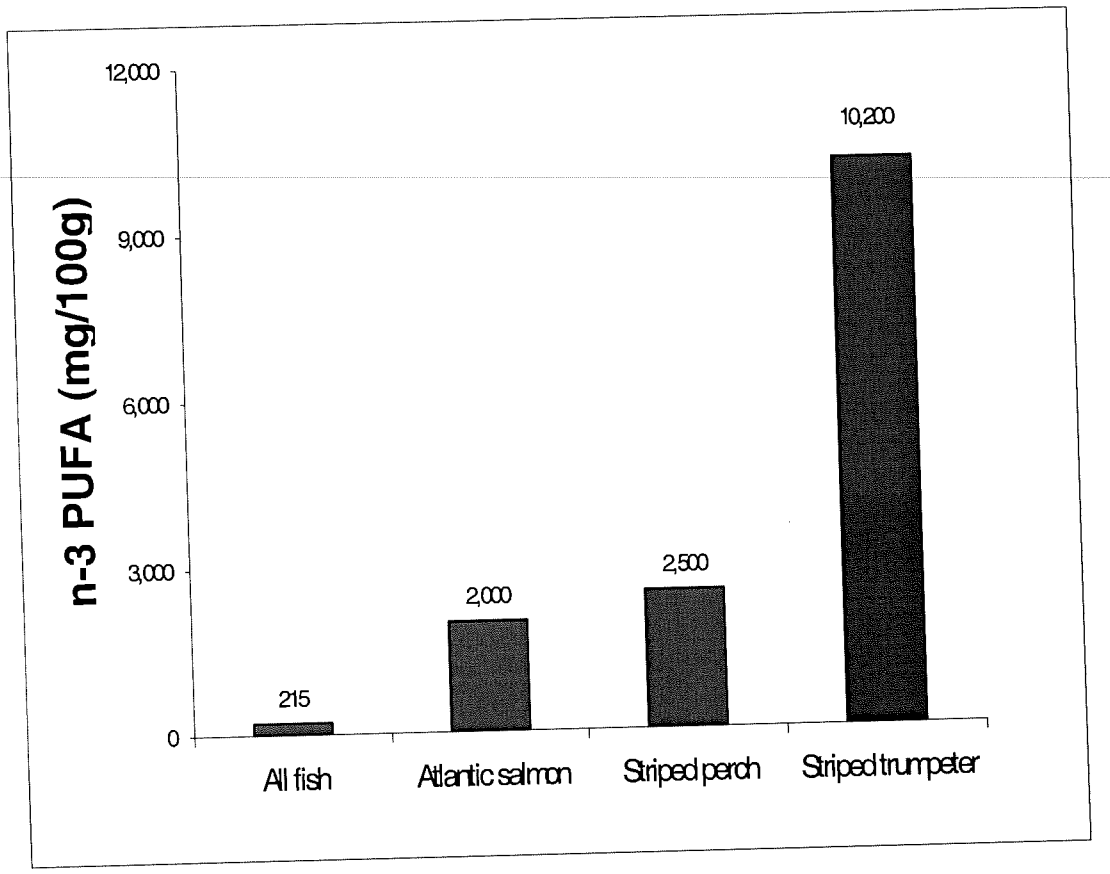


Figure 14.1. Omega-3 PUFA content of striped trumpeter, other farmed species (Atlantic salmon and striped perch), and the mean value for wild-caught Australian finfish (Nichols *et al.* 1998b).

15. Oil Composition of Tasmanian Farmed Seahorses

Results in this chapter were provided as a report to Seahorse Aquaculture Pty Ltd.
Nichols, P., Elliott, N. and Mooney, B. D. (2000) Oil composition of Tasmanian seahorses. Internal Report 2000-CMR5.

15.1 Summary of Results

The oil content and composition, and fatty acid composition, were determined for samples of cultured Tasmanian seahorses (*Hippocampus abdominalis*, dried and frozen juveniles, male and female broodstock) obtained from the Seahorse Australia Pty Ltd facility at Beauty Point. Analytical methods used were thin layer chromatography – flame ionization detection (TLC-FID) and gas chromatography (GC) / GC-mass spectrometry (GC-MS). Sample work-up and analytical methods were as described in Nichols *et al.* (1994). This study was performed as part of an FRDC-funded investigation (1999/331) of the oil composition of Australian seafoods.

Total oil (lipid) content of the seahorse samples varied from 0.6 to 2.2%, with the highest oil content observed for the dried sea horse product (Table 15.1). The frozen sea horse and broodstock samples were supplied and analysed as undried material. Phospholipid (PL, 48-62% of total lipid) was the major lipid class present in all samples, followed by triacylglycerol (TAG, 17-31%; male and female broodstock; frozen juveniles). Large variations within sample replicates were apparent for both TAG and PL. In the dried sea horse sample, TAG levels were markedly lower at 6%, and free fatty acid (FFA) was elevated compared to the other samples (33% versus 3-15%). The higher FFA levels are consistent with the conversion of PL and TAG to FFA during drying and/or storage. Sterol, mainly cholesterol based on GC analysis, accounted for 8-11% of the total oil in all samples.

The major fatty acids in all seahorse samples were: 18:1ω9c (oleic acid), 16:0 (palmitic acid), 22:6ω3 (docosahexaenoic acid, DHA), 18:0 (stearic acid), 20:4ω6 (arachidonic acid, AA), 20:5ω3 (eicosapentaenoic acid, EPA) and 18:2ω6 (linoleic acid, LA) (Table 15.2). Polyunsaturated fatty acids (PUFA) were dominant in all samples (31-39%), with similar levels of monounsaturated fatty acids (MUFA, 35-39%) and slightly lower levels of saturated fatty acids (SAT, 26-30%). Variations in fatty acid composition were apparent between the four samples. For example, the broodstock samples (both male and female) contained lower relative (%) levels of EPA and DHA, and the frozen juveniles contained higher relative levels of LA. Such differences may be due to variations in diet, and/or environmental and other factors.

Considerable interest exists in the levels of the long-chain (C_{20} and C_{22}) omega-3 LC-PUFA in seafood and marine oil products. This is the result of well-documented nutritional benefits of these unique PUFA. They help against coronary heart disease, high blood pressure, rhuematoid arthritis, and may also be beneficial against other disorders, including some forms of cancer. The LC-omega-3 PUFA are also important in larval development and survival for many aquaculture species. Most Australian seafood contains elevated levels of the long-chain omega-3 oils, in particular the beneficial DHA and EPA (Nichols *et al.* 1998a&b). The absolute concentrations of DHA and EPA in the dried and frozen

juvenile seahorse samples (omega-3 PUFA: dried seahorse, 388 mg/100g; frozen juveniles, 241 mg/100g; Table 15.3, Figure 15.1) compares favorably with concentrations found in Australian seafood (Nichols *et al.* 1998a&b).

The oil compositional results for cultured Tasmanian seahorses presented in this report represent to our knowledge the first such detailed compositional data for this species. It will be possible to change the oil profile by adjusting diet, and other factors including processing. Analyses of further samples of oil from this species may be beneficial to gain insight into compositional variations that may occur due to changes in diet, environment and other factors.

15.2 Acknowledgement

Patti Virtue and Mark Rayner kindly helped with sample collection and transport to Hobart from the Seahorse Aquaculture Pty Ltd Beauty Point facility.

Table 15.1. Lipid class composition (%) of Seahorse Australia Pty Ltd samples.
Data are the mean of 3 samples.

Lipid class	Percentage composition							
	Seahorse Dried (male)		Seahorse Frozen (juv)		Seahorse Broodstock (male)		Seahorse Broodstock (female)	
Wax ester	0.9	±0.6	0.3	±0.3	0.5	±0.7	0.6	±0.5
Triglyceride	6.0	±6.7	23.6	±40.2	31.0	±41.0	17.4	±21.6
Free fatty acid	32.6	±8.1	2.6	±1.5	11.7	±11.8	14.8	±11.4
Cholesterol	7.7	±1.3	11.4	±6.6	8.8	±7.6	9.6	±3.5
Phospholipid	52.8	±12.9	62.2	±34.8	48.0	±44.5	57.7	±30.0
Total lipid (%)	2.2	±1.1	1.2	±1.0	0.6	±0.8	0.7	±0.2

Frozen juvenile and broodstock samples supplied and analysed undried.

Table 15.2. Fatty acid composition (%) of Seahorse Australia Pty Ltd samples. Data are the mean of 3 samples.

Fatty acid	Percentage composition Seahorse Dried (male)		Seahorse Frozen (juv)		Seahorse Broodstock (male)		Seahorse Broodstock (female)	
14:1	1.3	±1.8	0.0	±0.0	1.8	±2.3	2.7	±3.5
14:0	4.0	±1.6	1.6	±0.4	4.4	±1.3	5.0	±1.3
a15:0	0.5	±0.2	0.9	±0.3	0.4	±0.1	0.5	±0.1
15:0	0.5	±0.1	0.3	±0.0	0.3	±0.0	0.4	±0.1
16PUFA	0.3	±0.1	0.2	±0.1	0.1	±0.0	0.1	±0.1
16:1ω9c	0.9	±0.1	0.9	±0.1	0.6	±0.1	0.8	±0.2
16:1ω7c	4.2	±0.6	1.7	±0.9	4.7	±1.9	5.4	±3.8
16:2	0.3	±0.3	0.1	±0.0	0.0	±0.1	0.2	±0.1
16:1ω5c	0.3	±0.0	0.1	±0.0	0.3	±0.2	0.3	±0.2
16:0	14.6	±1.1	10.0	±1.2	12.5	±0.0	14.6	±1.9
17:1/a17:0	0.6	±0.2	0.6	±0.4	0.4	±0.0	0.5	±0.1
17:0	0.7	±0.2	0.9	±0.1	0.6	±0.0	0.6	±0.1
18:3ω6	0.3	±0.0	0.4	±0.3	0.2	±0.0	0.2	±0.0
18:4ω3	0.8	±0.1	0.7	±0.9	0.1	±0.0	0.2	±0.2
18:2ω6 LA	4.8	±2.9	9.3	±2.6	3.7	±0.2	2.8	±0.2
18:3ω3	0.4	±0.6	0.0	±0.0	0.4	±0.2	0.7	±0.4
18:1ω9c	18.8	±1.1	22.7	±7.1	18.5	±0.1	19.1	±3.5
18:1ω7c	4.6	±0.3	4.6	±2.0	3.4	±0.7	4.6	±1.2
18:1ω5c	0.2	±0.1	0.0	±0.0	0.3	±0.1	0.3	±0.2
18:0	7.3	±0.9	9.9	±3.8	8.6	±2.6	7.4	±3.1
20:4ω6 AA	6.6	±3.4	4.3	±2.0	12.1	±0.2	9.7	±3.0
20:5ω3 EPA	5.3	±2.4	6.3	±3.7	3.1	±0.1	2.8	±1.2
20:3ω6	0.3	±0.0	0.1	±0.0	0.2	±0.1	0.2	±0.0
20:4ω3 ETA	0.4	±0.0	0.4	±0.4	0.2	±0.1	0.2	±0.1
20:2ω6	0.4	±0.2	0.3	±0.2	0.2	±0.1	0.7	±0.4
20:1ω9c	1.6	±0.4	1.0	±0.6	1.6	±0.8	1.8	±0.7
20:1ω7c	0.4	±0.2	0.2	±0.1	0.5	±0.4	0.5	±0.3
20:0	0.4	±0.1	0.6	±0.4	0.5	±0.0	0.4	±0.1
21PUFA	0.1	±0.2	0.1	±0.2	0.0	±0.0	0.0	±0.0
22:5ω6	0.4	±0.0	0.5	±0.2	0.4	±0.1	0.3	±0.2
22:6ω3 DHA	11.2	±2.4	12.2	±3.1	9.3	±3.0	6.8	±5.8
22:4ω6	1.2	±0.8	0.5	±0.3	2.5	±0.2	2.8	±0.8
22:5ω3 DPA	2.5	±0.4	3.4	±0.6	2.3	±0.7	2.3	±1.2
22:3ω3	0.2	±0.0	0.0	±0.0	1.1	±0.4	0.6	±0.4
22:1ω9c	0.6	±0.3	0.7	±0.5	0.7	±0.1	0.8	±0.3
22:1ω7c	0.4	±0.2	0.4	±0.3	0.5	±0.1	0.7	±0.1
22:0	0.5	±0.1	0.9	±0.6	0.5	±0.2	0.4	±0.2
24:1	1.1	±0.1	1.6	±1.1	1.8	±0.5	1.5	±0.9
24:0	0.3	±0.1	0.5	±0.4	0.4	±0.2	0.2	±0.2
Sum SAT	29.1	±1.5	26.0	±6.8	28.5	±1.4	29.9	±0.1
Sum MUFA	35.4	±2.5	35.1	±9.0	35.5	±5.7	39.3	±12.6
Sum PUFA	35.5	±4.0	38.9	±3.2	36.1	±4.3	30.8	±12.5
sum ω3	20.7	±4.6	23.1	±1.7	16.5	±3.7	13.6	±8.7
sum ω6	14.0	±1.2	15.4	±4.6	19.4	±0.6	16.8	±3.8

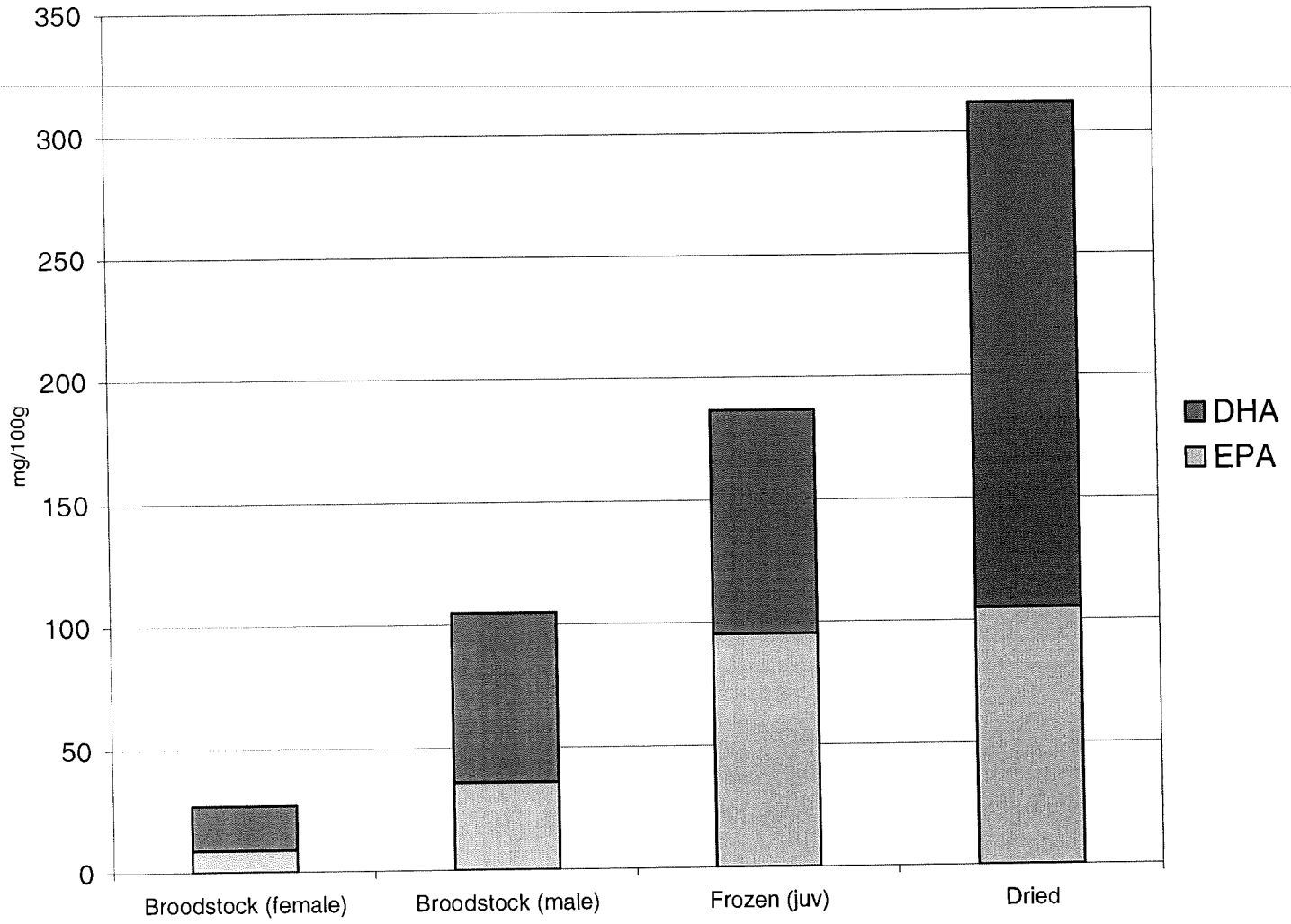
Frozen juvenile and broodstock samples supplied and analysed undried.

Table 15.3. Fatty acid content (mg/100g) of Seahorse Australia Pty Ltd samples.
Data are the mean of 3 samples.

	Fatty acid content (mg/100g)							
	Seahorse Dried (male)		Seahorse Frozen (juv)		Seahorse Broodstock (male)		Seahorse Broodstock (female)	
Sum SAT	501.9	±256.4	195.2	±237.8	45.8	±17.8	127.1	±95.4
Sum MUFA	608.1	±300.5	421.9	±629.0	59.8	±34.4	187.1	±179.2
Sum PUFA	641.8	±415.3	355.7	±478.1	57.1	±18.6	111.1	±45.7
sum ω3	388.3	±286.0	240.7	±341.3	25.6	±5.8	43.9	±6.4
sum ω6	239.0	±115.5	110.7	±130.2	31.4	±12.8	65.2	±37.6

Frozen juvenile and broodstock samples supplied and analysed undried.

Figure 15.1. DHA and EPA content of Seahorse Australia Pty Ltd samples.
Frozen juvenile and broodstock samples were supplied and analysed undried.



16. Oil Content and Composition of Farmed Murray cod and Striped perch

Results in this chapter were provided as a report to Australian Aquaculture Products.
Mooney, B., Nichols, P. Elliott, N. (2001) Oil content and composition of wild Murray cod. Report 2001 CMR/MP3.

16.1 Introduction

The oil content and composition, and fatty acid composition were determined for samples of farmed Murray cod (*Maccullochella peelii*) and Striped perch (*Scortum barcoo*). Samples (n=2 or 3 for each) consisted of frozen flesh and belly flap, received from Australian Aquaculture Products in July 2001.

Analytical methods used were thin layer chromatography – flame ionisation detection (TLC-FID) and gas chromatography (GC)/GC-mass spectrometry (GC-MS). Sample work-up and analytical methods were as described in Nichols *et al.* (1994, 1998a&b).

This study was performed as part of an FRDC-funded investigation (1999/331) of the oil composition of Australian seafood.

16.2 Results

Oil content and class composition

Total oil (lipid) content of the aquacultured Murray cod samples were 0.8%, which is similar to wild-caught specimens (0.7%) (Table 16.1). The oil content of farmed Striped perch was markedly high at 16.3%. The average for Australian seafood is around 1% (Nichols *et al.* 1998a). The belly flap (fat deposit) in both species contained a very high oil content - Murray cod 82.8% and Striped perch 88.7% (Table 16.1).

Aquacultured and wild Murray cod had a similar lipid class composition with phospholipid (80-84%) and triglyceride (11-13%) as the major lipids. Triglyceride was the major lipid class in Jade perch samples (98.0%). The belly flap of Murray cod and Striped perch was also dominated by triglyceride (98-99%). High oil content correlated well with high triglyceride levels, which is in agreement with previous findings of FRDC-funded research on the oil composition of Australian seafood (Nichols *et al.* 1998a&b).

Fatty acid composition

The major fatty acids in farmed Murray cod samples were 16:0 (24%), DHA (21%), EPA (10%), 18:1ω9 (11%) and 18:0 (9%) (Table 16.2). These 5 components accounted for 75% of the total

fatty acids. Compared to wild Murray cod, the farmed cod contained higher EPA and 16:0, with lower levels of 18:1ω9. Similar levels of DHA were observed between wild and farmed specimens. Farmed Striped perch contained 16:0 (22%), 18:1ω9 (20%), DHA (10%), 16:1ω7 (8%) and EPA (7%). These accounted for 67% of the total fatty acids. Belly flap samples from both species were dominated by 16:0 (21-23%), 18:1ω9 (17-18%), 16:1ω7 (9%) and DHA (6-10%).

Polyunsaturated fatty acids (PUFA) (44-45%) were dominant over saturated fatty acids (SAT) (29-36%) and monounsaturated fatty acids (MUFA) (19-26%) in wild and farmed Murray cod. However, the farmed cod had a greater omega-3/omega-6 ratio. Farmed Striped perch had higher levels of SAT (35%) and MUFA (34%), with lower levels of PUFA (31%). The content of omega-3 fatty acids (in mg/ 100g) was similar in wild and farmed Murray cod (172-177 mg/ 100g). Aquacultured Striped perch contained 3870 mg/ 100g omega-3 fatty acids. The amount of omega-3 PUFA in belly flap samples from both species is also very high (11290-23720 mg/ 100g).

16.3 Discussion

There is ever increasing interest in the long chain polyunsaturated fatty acids found in seafood and marine oil products. The nutritional benefits for humans is well documented (Nichols *et al.* 1998a& b), and most Australian seafood contain elevated levels of long chain omega-3 fatty acids, in particular DHA and EPA.

The observed omega-3 PUFA content value in farmed Striped perch (3870mg/100g) is extremely high. This value represents to our knowledge one of the highest reported levels to date of omega-3 PUFA in Australian seafood (mean 210mg/100g, range 30 to 1100mg/100g; Nichols *et al.* 1998a & b). Relative to commercially available fish oil supplements, one 100g serving of the oil-rich Striped perch would provide the equivalent of ten 1000mg capsules of fish oil containing omega-3 PUFA. The possible availability of such a high dose of omega-3 PUFA in a meal may be of considerable interest to medical and health practitioners seeking to treat a range of disorders with omega-3 PUFA. The high oil level (>16%), however, may not be entirely acceptable for all forms of processing or to some consumers from a taste/texture perspective.

Farmed Murray cod had a similar oil content and fatty acid composition to wild specimens. The oil composition results for Murray cod and Striped perch on culture diets show these species as good and excellent sources respectively of omega-3 fatty acids. These compositions may be further manipulated via suitably formulated diets.

Belly flap samples from both Murray cod and Striped perch were very high in oil, and represent a good source of omega-3 fatty acids. This waste product has the potential to be processed for its' oil and PUFA content.

Table 16.1. Lipid class content and composition and lipid content (%) of Murray cod and Striped perch.

Lipid class	Murray cod			Striped perch	
	Wild	Aquaculture	Belly flap	Aquaculture	Belly flap
%					
Wax ester	0.0	1.0	0.0	0.0	0.0
Triglyceride	13.0	11.1	99.5	98.0	97.9
Free fatty acid	2.6	1.4	0.3	0.1	0.9
Cholesterol	4.4	3.0	0.0	0.2	0.8
Phospholipid	80.0	83.6	0.1	1.7	0.4
mg/ 100 g					
Wax ester	0	7	0	0	0
Triglyceride	93	78	82460	16020	86850
Free fatty acid	18	10	2	20	8
Cholesterol	31	23	0	37	7
Phospholipid	563	633	1	250	3
Total lipid (%)	**0.7**	**0.8**	**82.8**	**16.3**	**88.7**

Table 16.2. Percentage composition of main individual fatty acids and fatty acid classes in Murray cod and Striped perch, and content of omega-3 fatty acids (mg wet weight) in 100g of tissue.

Fatty acid	Murray cod			Striped perch	
	Wild	Aquaculture	Belly flap	Aquaculture	Belly flap
14:0	1.0	1.8	8.3	6.1	6.5
16:1ω7	4.3	2.6	9.2	7.8	8.7
16:0	19.3	24.1	22.6	21.9	21.0
18:1ω7	3.1	2.5	4.4	2.8	3.2
18:1ω9	14.6	11.4	17.3	20.2	17.7
18:2ω6	4.4	1.8	3.5	2.6	2.7
18:4ω3	0.9	0.8	2.2	2.2	2.4
18:0	7.1	8.6	5.3	4.3	4.3
20:1	1.6	0.7	1.9	1.2	1.1
20:4ω6	5.2	3.6	0.6	0.6	0.5
20:5ω3 EPA	6.1	10.4	5.9	7.4	8.2
22:5ω3	4.1	4.0	3.3	3.9	4.3
22:5ω6	0.7	1.0	0.2	0.2	0.2
22:6ω3 DHA		21.8	20.5 6.1	9.7	10.3
Total SAT	29.0	36.3	39.7	34.5	34.6
Total MUFA	25.8	19.2	34.8	33.7	32.3
Total PUFA	45.3	43.8	25.5	30.8	33.1
Total ω3	33.2	36.1	18.5	24.1	26.2
Total ω6	10.6	7.0	4.9	4.2	4.1
ω3/ω6	3.1	5.2	3.8	5.8	6.3
ω3 (mg/100g)	172	177	11290	3870	23720

17. The Effect of Processing on the Oil Content and Composition of Seafood I. Cooking and Value-added Soups

Results in this chapter were provided as a report to Mures.
Mooney, B. Elliott. N. and Nichols, P. (2001) The Effect of Processing on the Oil Content and Composition of Seafood. I. Cooking and Value-added Soups. Report 2001-Mures1. Report prepared for Mures Seafood.

17.1 Summary

Three Australian fish – blue eye, gummy shark and dogfish - were examined to determine the effect of different cooking procedures on oil content and composition of the fillets. Cooking methods were: grilling, pan and deep frying, steaming and microwaving. Emphasis was placed on the levels of the beneficial long-chain (C_{20} and C_{22}) omega-3 polyunsaturated fatty acids (LC-PUFA), in particular eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3).

The flesh of the three fish species varied in oil content: blue eye, 1.4%; gummy shark, 0.9%; dogfish, 3.5%. The LC-PUFA, predominantly DHA and EPA, accounted for 35-50% of the total fatty acids in fresh fish. DHA was the dominant component at 22-27% in all species. On an absolute basis, the omega-3 LC-PUFA content was: blue eye, 244 mg/100 g; gummy shark, 153 mg/100 g; dogfish, 660 mg/100 g. The three fish are excellent sources of the beneficial omega-3 LC PUFA. Of note, the levels found for the less sought-after dogfish make it a particularly good source of the omega-3 LC PUFA.

The lower value dogfish is a by-catch of other southern fisheries. Limited oil composition data is available for this species. In addition to the main oil classes present, the less common oil class diacylglyceryl ether (DAGE) was present at 7% of the total oil. Interest exists in DAGE-containing oils as nutraceuticals; a 200 g serving of dogfish would supply 0.63 g of DAGE.

The oil content of the fillets of all three species varied depending on the method of cooking. With microwave or steam cooking, oil and omega-3 LC-PUFA content and composition was generally similar to fresh fish. Oil content of each species increased with the other cooking methods, with highest oil content occurring for the deep-fried fish, followed by the pan fried and grilled fish. The higher oil content observed is consistent with uptake of the particular cooking oil by the fillet during cooking. As noted for steaming and microwave cooking, the content of the omega-3 LC-PUFA, DHA and EPA, was largely not affected by these forms of cooking.

Three commercially-available packaged soups derived partly from seafood by-products were examined for oil content and composition. The soups are sold in ready to serve packs under the labels: Prawn bisque, Smokey fish chowder and Le Provencal. Oil content of the soups were: Prawn bisque (2.3%), Smokey fish chowder (1.1%) and Le Provencal (0.1%). The LC-PUFA, predominantly DHA, EPA and AA accounted for 1-12% of the total fatty acids in the soups. On an absolute basis, the omega-3 LC-PUFA content was - Prawn bisque, 18 mg/100 g; Smokey fish chowder, 33 mg/100 g and Le Provencal, 4 mg/100 g. These values are lower than the average

value for Australian seafood, and for the particular species used in the soups. However, in comparison to other food groups such as veal, pork, lamb chicken and turkey, the Prawn bisque and Smokey fish chowder provide similar or higher levels of the beneficial omega-3 LC-PUFA.

17.2 Introduction

There is increasing nutritional interest in the long-chain (C_{20} and C_{22}) omega-3 polyunsaturated fatty acids (LC-PUFA). In particular, eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3) in seafood and marine oil products receive special attention. This is the result of well-documented nutritional benefits of these unique PUFA. They help against coronary heart disease, high blood pressure, rheumatoid arthritis, and may also be beneficial against other disorders, including some forms of cancer, depression and other neural illnesses. Most Australian seafood contains elevated levels of the long-chain omega-3 oils, including the beneficial DHA and EPA. The oil composition of 200 species of Australian seafood is available in the Guide *Seafood the Good Food* and in an accompanying FRDC report (Nichols *et al.* 1998a&b).

Whilst considerable data now exists on the oil composition of Australian seafood, limited information is available on the effect of processing, including cooking and other forms of value-adding, on the oil profiles. In this study we report on the oil content of two fish species commonly consumed in south-east Australia, blue-eye trevalla and gummy shark, and a third less sought-after species, dogfish. Experiments were conducted to examine the effect of various forms of cooking (pan-fry, deep fry, grill, steam, microwave) on the oil composition of the three species. In addition, three commercially-available packaged soups containing some seafood by-products were also examined.

This study was performed as part of a Fisheries Research and Development Corporation (FRDC) supported investigation (1999/331) of the oil composition of Australian Seafood.

17.3 Materials and Methods

Samples

Three specimens of each of blue eye trevalla, gummy shark and dogfish were provided by Mures Fish Centre. All cooking was performed in a commercial kitchen by a qualified chef at the Mures Upper Deck Restuarant, and involved triplicate samples (one from each specimen) for each species and cooking method. Approximately 100 g portions of fish were used. The modes of cooking were pan fry, deep fry, grill, steam and microwave. Cooking oils used were: peanut oil, deep fry; cottonseed oil, grill and pan fry. After cooking, all samples were placed in separate bags and stored frozen prior to analysis.

Soup samples were obtained from sealed containers of the three varieties of pre-packed Mures Gourmet soups: Prawn bisque, Smokey fish chowder and Le Provencal. Soups provided by Mures Fish Centre were analysed in duplicate.

Oil analyses

Oil (lipid) analyses were conducted using methods developed during FRDC projects 1995/122 and 1999/331. Full details are provided in reports prepared for FRDC (Nichols *et al.* 1998a&b), with the methods also published in the peer-reviewed literature (e.g. Bakes *et al.* 1995, Nichols *et al.* 1998c). Briefly, samples were extracted using a single phase Bligh and Dyer (1959) procedure. Oil yield was determined gravimetrically. An aliquot of the oil was analysed by TLC-FID to determine lipid class composition. Fatty acid profiles were obtained by capillary GC and GC-MS analysis following transmethylation of an aliquot of the extracted oil.

17.4 Results and Discussion

Fresh fish

Blue eye trevalla

Fresh blue eye contains 1.4% oil, with polar lipid and triglyceride as the main oil classes (84% and 14% respectively, Table 17.1). The omega-3 LC-PUFA, predominantly DHA and EPA, accounted for 35% of the total fatty acids, with total PUFA at 41%. DHA was the dominant component at 27% (Table 17.2). On an absolute basis, the omega-3 LC-PUFA content was 244 mg/100 g (Table 17.3). This value is slightly higher than the average for Australian fish (210 mg/100 g; Nichols *et al.* 1998a&b). The oil class and fatty acid profiles are generally similar to those reported in *Seafood the Good Food.*

Gummy shark

Gummy shark fillet contained slightly lower oil content (0.9%) compared to blue eye, with polar lipid as the main oil class (97% of total oil, Table 17.1). The LC-PUFA, predominantly DHA, EPA and arachidonic acid (AA, 20:4ω6) accounted for 50% of the total fatty acids. DHA was again the dominant component at 22% (Table 17.4). On an absolute basis, the omega-3 LC-PUFA content was 153 mg/100 g (Table 17.5), lower than measured for blue eye and slightly lower than the average for Australian fish. The oil class profile was similar to previous research findings, although the fatty acid profile differed from that reported in *Seafood the Good Food.* A lower content of the omega-3 LC-PUFA occurred in this study, together with higher levels of AA. These differences may be due to variations in feeding behaviour, environmental, genetic and other factors.

Dogfish

The lower-commercial value dogfish is a by-catch of other southern fisheries. Limited oil composition data is available for flesh from this species.

Fresh dogfish contains 3.5% oil, markedly higher than the other two species. Equal proportions of polar lipid and triglyceride were present (Table 17.1). In addition to the main oil classes

present, the less common oil class - diacylglyceryl ether (DAGE) - was present at 7% of the total oil.

Interest exists in DAGE-containing oils as nutraceuticals. Capsules of DAGE oils derived from the livers of deep-sea sharks are marketed as neutraceuticals for human consumption. Based on the findings of this study, dogfish fillets represent an additional source of the DAGE oils. The health benefits reported to be associated with shark liver oils include an enhanced immune system, antibacterial activity, and possible regression of tumour growth and radiation induced damage. Therapeutic doses of these oils are of the order of 1 to 5 g per day. In comparison, a 200 g serving of dogfish would supply 0.63 g of DAGE.

The LC-PUFA, predominantly DHA, EPA, docosapentaenoic acid (DPA, 22:5ω3) and AA accounted for 40% of the total fatty acids in dogfish (Table 17.6); omega-3 LC PUFA represented 32%. DHA was the dominant component at 24%. On an absolute basis, the omega-3 LC-PUFA content was 660 mg/100 g (Table 17.6). This value is markedly higher than measured for the other two higher value table species, and is also significantly greater than the average content for Australian fish.

Cooked fish

Oil content of all species varied depending on the method of cooking used. Blue eye and gummy shark were cooked in five ways: grill, steam, microwave, and pan and deep fry. Dogfish was deep fried only.

Steamed and microwaved

With cooking by microwave or steaming, oil and omega-3 LC-PUFA content and composition for blue eye and gummy shark was similar to fresh fish (Tables 17.1-17.5, Figure 17.1 and 17.2).

Pan and deep frying

Oil content of blue eye and gummy shark increased with pan and deep frying (Tables 17.3 and 17.5). The oil content for the deep-fried blue eye was 5.3%, and pan fried 2.3%, compared to 1.4% in fresh fish. Oil content of the deep-fried and pan-fried gummy shark were both 3.8%, up from 0.9% in fresh fish. Deep-fried dogfish contained the highest oil content of any samples examined in this study (7.1%, up from 3.5%, Table 17.6).

The higher oil content observed in the deep-fried and pan-fried fish is consistent with uptake of cooking oil by the fillet during frying. The relative uptake differed between blue eye and gummy shark, with for example higher uptake of oil by gummy shark during pan frying.

The content of the main omega-3 LC-PUFA - DHA and EPA - was largely not affected by deep frying and pan frying (blue eye range 260-296 mg/100 g, Table 17.3; gummy shark range 200-207 mg/100 g, Table 17.5; dogfish 735 mg/100 g, Table 17.6; Figures 17.1 and 17.2). The differences that were observed in the absolute amount of these beneficial PUFA can be attributed to within-sample variation, and also due to loss of water during cooking.

Examination of the fatty acid profiles expressed in percent form shows that the deep-fried and pan-fried fish contained higher relative levels of vegetable oil derived components. As noted with the higher oil content observed with these cooking methods, this feature is due to incorporation of cooking oil by the fillet. The deep-fried fish all contained higher levels of the omega-6 PUFA linoleic acid (LA, 18:2ω6, blue eye 43%, gummy shark 40%, dogfish 25%, Tables 17.2, 17.4 and 17.6) consistent with the use of peanut oil. Oleic acid (OA, 18:1ω9c) was elevated in the pan fried fish (blue eye 41%, gummy shark 50%, Tables 17.2 and 17.4). OA (62%) is the major fatty acid in the cottonseed oil used for pan-frying, with LA (53%) dominant in peanut oil which is used for deep frying (Table 17.6).

Grilling

Grilling fish also saw a small increase, relative to fresh fish, in oil content as the fish were brushed with cottonseed oil. Oil contents observed were: blue eye, 1.9% and gummy shark, 1.3% (Table 17.1).

The content of the main omega-3 LC-PUFA - DHA and EPA - was not negatively affected by grilling; for both species an increase was observed (blue eye 324 mg/100 g, Table 17.3; gummy shark 251 mg/100 g, Table 17.5), which is attributed to a loss of water during grilling.

Incorporation of cooking oil during grilling resulted in an increase in oleic acid in blue eye (OA 36%) and gummy shark (OA 25%).

Soups

Three soups derived partly from seafood by-products were examined for oil content and composition. The soups are sold in ready to serve packs under the labels: Prawn bisque, Smokey fish chowder and Le Provencal.

Oil content of the soups varied twenty fold - Prawn bisque (2.3%), Smokey fish chowder (1.1%) and Le Provencal (0.1%) (Table 17.7). The oil profiles of the Prawn bisque and Smokey fish chowder were dominated by triglyceride, with Le Provencal containing equal portions of triglyceride and polar lipid.

The LC-PUFA, predominantly DHA, EPA and AA, accounted for 1-12% of the total fatty acids (Table 17.8). DHA was the dominant LC-PUFA in Smoky fish chowder and Le Provencal. On an absolute basis, the omega-3 LC-PUFA content was - Prawn bisque, 18 mg/100 g; Smokey fish chowder, 33 mg/100 g and Le Provencal, 4 mg/100 g. These values are lower than measured for the fresh fish examined in this study, and also lower than the average value for Australian fish. However, in comparison to other food groups such as veal, pork, lamb chicken and turkey, the Prawn bisque and Smokey fish chowder provide similar or higher levels of the beneficial omega-3 LC-PUFA.

A number of functional food items are now available that offer alternate souces of the omega-3 LC-PUFA. For example, "HiQ" bread containing DHA derived from tuna oil has been marketed

since 1999. A single serving of the "HiQ" bread provides 36 mg of the beneficial LC-PUFA, ie. levels similar to that provided by the Prawn bisque and Smokey fish chowder soups.

17.5 Conclusion

The current study represents, to our knowledge, one of the first detailed comparative studies of the effect of various cooking processes and other forms of value-adding on the oil content and composition across a range of Australian species.

Importantly no loss of the beneficial omega-3 LC-PUFA was observed for any of the forms of cooking examined. An increase in oil content and levels of specific components was seen with frying and grilling, reflecting uptake of vegetable oil components. In terms of nutritional value, whilst the content of omega-3 LC-PUFA did not decrease, the ratio of omega-3 to omega-6 fatty acids did, however, decrease markedly with frying and grilling. Such changes in oil profiles will need further nutritional evaluation. The use of cooking oils containing lower levels of omega-6 PUFA may be worthy of consideration.

Soup products derived partly from seafood by-products can also provide omega-3 LC-PUFA. As a variety of seafood by-products can contain high concentrations of the beneficial omega-3 LC-PUFA (e.g. salmon frames contain higher levels omega-3 LC-PUFA than occurs in fillet, see chapter 12), scope to further increase the level of beneficial oils in soups or stocks may exist.

17.6 Acknowledgement

Mures Fish Centre kindly provided fish and soup analysed in this study. We thank Jill and George Mure and Paul Lynch for their help with the cooking performed in the Mures Upper Deck Restuarant, and for their ongoing interest in and support of the project.

Table 17.1 Lipid class composition (%) and content (mg/100g) of fresh and cooked fish, and cooking oils

	Gummy shark					
mg/100g	Fresh	Pan Fry	Deep Fry	Steam	Grill	Microwave
Wax ester	0 ±0	10 ±1	1 ±2	0 ±0	1 ±2	1 ±1
Triglyceride	5 ±1	2709 ±2149	2825 ±1446	10 ±9	234 ±237	19 ±17
Free fatty	2 ±1	8 ±8	16 ±11	3 ±4	3 ±3	4 ±4
Cholesterol	16 ±2	24 ±7	41 ±7	14 ±2	22 ±2	16 ±4
Phospholipid	847 ±27	1055 ±182	918 ±371	872 ±87	1062 ±121	955 ±115
Total	870 ±29	3807 ±2048	3801 ±1058	899 ±93	1322 ±140	994 ±111
%Total lipid	0.9 ±0.0	3.8 ±2.0	3.8 ±1.1	0.9 ±0.1	1.3 ±0.1	1.0 ±0.1
%						
Wax ester	0.0 ±0.0	0.3 ±0.1	0.0 ±0.1	0.0 ±0.0	0.1 ±0.2	0.1 ±0.1
Triglyceride	0.6 ±0.1	64.4 ±18.7	70.4 ±21.4	1.1 ±0.9	16.7 ±15.5	2.0 ±1.9
Free fatty	0.2 ±0.1	0.2 ±0.2	0.5 ±0.5	0.4 ±0.4	0.2 ±0.2	0.3 ±0.4
Cholesterol	1.8 ±0.2	0.8 ±0.4	1.2 ±0.5	1.6 ±0.2	1.7 ±0.3	1.6 ±0.3
Phospholipid	97.4 ±0.1	34.2 ±18.1	27.9 ±20.4	97.0 ±1.5	81.3 ±15.2	95.9 ±1.4
	Blue eye					
mg/100g	Fresh	Pan Fry	Deep Fry	Steam	Grill	Microwave
Wax ester	0 ±0	6 ±5	14 ±8	1 ±1	4 ±4	3 ±5
Triglyceride	204 ±138	1287 ±345	4461 ±1620	69 ±68	741 ±375	410 ±456
Free fatty	4 ±3	5 ±4	17 ±3	3 ±1	4 ±2	4 ±1
Cholesterol	24 ±12	26 ±14	49 ±11	21 ±4	28 ±19	20 ±6
Phospholipid	1162 ±447	942 ±455	719 ±163	998 ±144	1098 ±412	1058 ±67
Total	1395 ±526	2266 ±740	5260 ±1755	1091 ±208	1874 ±792	1494 ±508
%Total lipid	1.4 ±0.5	2.3 ±0.7	5.3 ±1.8	1.1 ±0.2	1.9 ±0.8	1.5 ±0.5
%						
Wax ester	0.0 ±0.0	0.2 ±0.1	0.3 ±0.1	0.1 ±0.1	0.1 ±0.1	0.0 ±0.1
Triglyceride	14.1 ±8.6	58.1 ±11.5	84.4 ±2.9	5.7 ±4.8	26.8 ±14.4	8.5 ±9.0
Free fatty	0.3 ±0.1	0.2 ±0.1	0.3 ±0.1	0.3 ±0.1	0.2 ±0.0	0.2 ±0.1
Cholesterol	1.7 ±0.2	1.1 ±0.4	1.0 ±0.1	2.0 ±0.7	1.5 ±0.4	1.7 ±0.6
Phospholipid	83.9 ±8.4	40.4 ±11.0	14.1 ±2.8	92.0 ±4.2	71.4 ±14.7	89.5 ±8.5

Table 17.1
continued

	Dogfish (Fresh)	Dogfish (Deep fry)
mg/100g		
Wax ester	0 ±0	0 ±0
Diacylglyceryl ether	316 ±391	249 ±189
Triglyceride	1968 ±2175	6049 ±1282
Free fatty	1 ±2	15 ±26
Cholesterol	19 ±4	29 ±11
Phospholipid	1170 ±155	723 ±78
Total	3473 ±2554	7064 ±1372
%Total lipid	3.5 ±2.6	7.1 ±1.4
%		
Wax ester	0.0 ±0.0	0.0 ±0.0
Diacylglyceryl ether	6.6 ±5.2	3.3 ±2.3
Triglyceride	44.7 ±25.1	85.5 ±3.3
Free fatty	0.1 ±0.2	0.2 ±0.3
Cholesterol	0.8 ±0.7	0.4 ±0.1
Phospholipid	47.8 ±29.5	10.6 ±2.9

	Grilling Oil	Deep fry oil
%		
Wax ester	0.2	0.0
Triglyceride	98.8	98.7
Free fatty	0.1	0.2
Cholesterol	0.1	0.3
Phospholipid	0.8	0.8

Table 17.2. Fatty acid composition (%) of fresh and cooked blue eye

	% Fresh	Pan Fry	Deep Fry	Steam	Grill	Micro wave
14:0	1.5 ±0.6	0.5 ±0.2	0.7 ±0.1	0.6 ±0.3	0.4 ±0.2	0.8 ±0.4
16:1ω7	0.8 ±0.1	0.5 ±0.1	0.6 ±0.1	0.8 ±0.4	0.5 ±0.2	0.9 ±0.7
16:0	18.3 ±1.8	10.2 ±1.5	21.5 ±0.2	18.5 ±1.4	11.4 ±0.7	18.2 ±1.1
18:2ω6	0.9 ±0.1	12.8 ±1.1	43.3 ±3.2	2.3 ±0.9	10.7 ±1.1	0.9 ±0.1
18:1ω9	13.6 ±2.3	41.1 ±4.5	18.8 ±0.1	12.1 ±3.1	35.7 ±2.6	11.3 ±5.0
18:1ω7	1.9 ±0.2	2.9 ±0.4	1.2 ±0.1	2.1 ±0.3	2.7 ±0.2	2.1 ±0.6
18:0	6.9 ±0.1	3.9 ±0.6	3.4 ±0.3	6.1 ±0.7	4.3 ±0.4	6.0 ±0.7
20:4ω6	2.8 ±0.6	1.2 ±0.4	0.5 ±0.1	2.9 ±0.6	1.6 ±0.4	3.0 ±0.8
20:5ω3	4.3 ±0.8	2.2 ±0.3	0.8 ±0.6	5.0 ±1.6	2.8 ±0.5	5.2 ±1.5
20:1ω9	8.6 ±2.2	2.5 ±1.2	0.6 ±0.2	3.2 ±0.8	2.5 ±0.9	4.0 ±1.4
22:6ω3	27.2 ±3.7	13.9 ±3.0	5.0 ±2.1	36.0 ±5.3	19.4 ±3.3	36.6 ±6.2
22:5ω3	2.0 ±0.2	1.1 ±0.1	0.3 ±0.2	2.5 ±0.5	1.3 ±0.1	2.6 ±0.2
22:1ω11	2.7 ±1.0	0.7 ±1.0	0.1 ±0.1	0.6 ±0.3	0.4 ±0.4	0.9 ±0.8
other	8.6	6.4	3.1	7.1	6.1	7.6
%						
Total SFA	27.9 ±1.5	15.8 ±1.9	26.5 ±0.3	26.5 ±1.7	17.5 ±1.1	26.3 ±1.4
Total MUFA	31.1 ±6.0	51.3 ±4.4	22.0 ±0.5	21.7 ±4.1	44.9 ±3.0	22.6 ±7.2
Total PUFA	41.0 ±5.0	32.8 ±2.6	51.6 ±0.8	51.8 ±4.1	37.5 ±2.5	51.2 ±5.7
Total w3	34.9 ±4.3	17.7 ±2.8	6.3 ±2.7	44.4 ±4.8	24.0 ±2.9	45.2 ±4.7
Total w6	5.6 ±0.7	15.0 ±0.5	44.1 ±3.0	7.1 ±1.2	13.4 ±0.7	5.8 ±1.3

Other includes
12:0,i14:0,14:1,i15:0,a15:0,15:0,16PUFA,i16:0,16:1w9,16:2,16:1ω5,i17:0,17:1ω8/a17:0,17:0,
18:3ω6,18:4ω3,18:3ω3,18:2,18:1ω5,20:3ω6,20:4ω3,20:2ω6,20:1ω11,20:1ω7,20:0,21PUFA,22:5ω6,
22:4ω6,22:3ω3,22:1ω9,22:1ω7,22:0,23PUFA,24PUFA,24:1,24:0

Table 17.3. Fatty acid content (mg/100g) of fresh and cooked blue eye

	mg/100g					
	Fresh	Pan Fry	Deep Fry	Steam	Grill	Microwave
14:0	11 ±7	8 ±3	31 ±10	4 ±3	6 ±4	6 ±4
16:1ω7	6 ±3	9 ±5	26 ±8	6 ±5	7 ±5	7 ±7
16:0	129 ±28	170 ±44	952 ±295	116 ±24	156 ±64	123 ±29
18:2ω6	6 ±2	228 ±113	1930 ±704	15 ±6	150 ±65	6 ±2
18:1ω9	101 ±43	736 ±387	832 ±255	80 ±40	501 ±223	84 ±56
18:1ω7	14 ±5	52 ±28	50 ±11	14 ±6	38 ±15	15 ±8
18:0	49 ±14	65 ±16	149 ±34	38 ±6	61 ±28	41 ±8
20:4ω6	20 ±4	20 ±2	19 ±2	18 ±1	21 ±10	20 ±5
20:5ω3	30 ±8	40 ±23	34 ±21	34 ±20	40 ±19	38 ±20
20:1ω9	64 ±34	43 ±20	24 ±9	20 ±6	38 ±26	29 ±14
22:6ω3	190 ±33	229 ±45	203 ±52	224 ±44	259 ±92	243 ±35
22:5ω3	15 ±4	18 ±6	14 ±5	16 ±5	18 ±7	17 ±5
22:1ω11	21 ±13	11 ±15	3 ±2	4 ±2	7 ±9	7 ±6
other	63	111	141	46	88	52
Total mg/100g	718 ±212	1742 ±715	4410 ±1322	634 ±170	1391 ±594	687 ±196
Total SFA	199 ±52	267 ±73	1166 ±347	167 ±36	244 ±109	179 ±106
Total MUFA	232 ±107	914 ±448	968 ±285	142 ±62	634 ±290	164 ±399
Total PUFA	287 ±57	561 ±194	2276 ±692	326 ±75	513 ±198	344 ±195
Total ω3	245 ±47	296 ±77	259 ±75	280 ±69	324 ±119	305 ±89
Total ω6	39 ±9	264 ±118	1965 ±707	44 ±9	187 ±80	38 ±113

Other includes
12:0,i14:0,14:1,i15:0,a15:0,15:0,16PUFA,i16:0,16:1ω9,16:2,16:1ω5,i17:0,17:1ω8/a17:0,17:0,18:3ω6, 18:4ω3,18:3ω3,18:2,18:1ω5,20:3ω6,20:4ω3,20:2ω6,20:1ω11,20:1ω7,20:0,21PUFA,22:5ω6,22:4ω6, 22:3ω3,22:1ω9,22:1ω7,22:0,23PUFA,24PUFA,24:1,24:0

Table 17.4. Fatty acid composition (%) of fresh and cooked gummy shark

	%					
	Fresh	Pan Fry	Deep Fry	Steam	Grill	Micro wave
14:0	0.5 ±0.1	0.2 ±0.1	0.6 ±0.1	0.4 ±0.0	0.3 ±0.1	0.4 ±0.1
16:1ω7	2.1 ±0.4	0.6 ±0.1	0.8 ±0.1	2.0 ±0.2	1.2 ±0.3	2.0 ±0.3
16:0	19.7 ±0.5	8.0 ±1.7	21.1 ±0.2	18.2 ±0.5	13.6 ±3.1	17.3 ±1.0
18:2ω6	1.7 ±0.2	16.7 ±3.2	39.9 ±6.1	1.8 ±0.7	7.8 ±5.4	2.3 ±1.0
18:1ω9	8.9 ±0.5	49.9 ±5.6	17.8 ±1.4	10.1 ±1.8	25.1 ±12.2	11.5 ±3.2
18:1ω7	4.6 ±0.1	3.9 ±0.4	1.9 ±0.4	4.7 ±0.5	4.2 ±0.6	4.6 ±0.3
18:0	9.7 ±0.2	3.8 ±0.7	4.4 ±0.6	9.0 ±0.2	6.7 ±1.3	8.5 ±0.4
20:4ω6	10.2 ±0.4	1.8 ±0.6	1.7 ±1.0	7.7 ±1.9	5.2 ±1.3	7.6 ±2.3
20:5ω3	8.2 ±2.0	1.8 ±1.1	1.5 ±0.6	7.8 ±1.1	4.5 ±0.9	7.7 ±0.9
20:1ω9	0.6 ±0.1	1.2 ±0.1	0.3 ±0.1	0.7 ±0.1	0.9 ±0.1	0.7 ±0.0
22:6ω3	21.7 ±1.9	6.3 ±3.9	5.3 ±3.3	24.1 ±2.8	19.5 ±8.1	24.7 ±2.6
22:5ω3	4.0 ±0.8	1.2 ±0.8	1.1 ±0.7	4.6 ±0.7	4.0 ±2.0	4.8 ±0.8
22:1ω11	0.0 ±0.0	0.1 ±0.2	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
other	8.1	4.4	3.6	8.7	6.9	7.9
Total SFA	31.4 ±0.6	13.1 ±2.4	27.1 ±1.0	29.3 ±0.4	22.2 ±4.6	27.7 ±1.5
Total MUFA	18.1 ±0.3	58.0 ±5.6	21.6 ±0.7	19.7 ±1.9	33.8 ±11.7	20.9 ±3.2
Total PUFA	50.5 ±0.3	28.9 ±3.5	51.4 ±0.3	51.0 ±2.1	44.0 ±7.1	51.4 ±1.8
Total ω3	35.2 ±0.3	9.6 ±5.7	8.1 ±4.7	37.9 ±3.7	28.7 ±11.2	38.3 ±2.8
Total ω6	15.2 ±0.1	19.3 ±2.4	42.3 ±5.0	12.9 ±3.6	15.3 ±4.3	13.0 ±2.6

Other includes
12:0,i14:0,14:1,i15:0,a15:0,15:0,16PUFA,i16:0,16:1ω9,16:2,16:1ω5,i17:0,17:1ω8/a17:0,17:0,
18:3ω6,18:4ω3,18:3ω3,18:2,18:1ω5,20:3ω6,20:4ω3,20:2ω6,20:1ω11,20:1ω7,20:0,21PUFA,22:5ω6,
22:4ω6,22:3ω3,22:1ω9,22:1ω7,22:0,23PUFA,24PUFA,24:1,24:0

Table 17.5. Fatty acid content (mg/100g) of fresh and cooked gummy shark

mg/100g	Fresh	Pan Fry	Deep Fry	Steam	Grill	Microwave
14:0	2 ±0	4 ±2	19 ±8	2 ±0	3 ±1	2 ±0
16:1ω7	9 ±1	15 ±8	22 ±7	10 ±2	11 ±2	11 ±2
16:0	85 ±4	201 ±101	642 ±259	94 ±11	121 ±21	99 ±14
18:2ω6	7 ±1	514 ±481	1266 ±650	9 ±4	75 ±63	13 ±5
18:1ω9	39 ±3	1475 ±1267	554 ±255	52 ±13	235 ±148	65 ±16
18:1ω7	20 ±1	104 ±68	55 ±14	24 ±2	38 ±2	26 ±4
18:0	42 ±2	99 ±57	129 ±40	46 ±4	60 ±6	48 ±5
20:4ω6	44 ±1	41 ±13	43 ±6	40 ±12	47 ±11	43 ±12
20:5ω3	35 ±8	41 ±15	39 ±3	40 ±5	40 ±4	43 ±2
20:1ω9	3 ±1	35 ±29	9 ±3	4 ±1	8 ±2	4 ±1
22:6ω3	94 ±12	129 ±27	135 ±24	123 ±8	171 ±60	142 ±31
22:5ω3	17 ±4	25 ±5	27 ±7	23 ±3	35 ±15	27 ±8
22:1ω11	0 ±0	6 ±10	0 ±0	0 ±0	0 ±0	0 ±0
other	35	123	105	45	62	45
Total mg/100g	434 ±20	2811 ±2133	3048 ±1252	512 ±50	906 ±145	570 ±64
Total SFA	136 ±6	335 ±185	818 ±316	150 ±17	198 ±30	158 ±20
Total MUFA	79 ±5	1699 ±1432	663 ±285	101 ±17	314 ±153	119 ±17
Total PUFA	219 ±11	777 ±518	1568 ±651	261 ±21	394 ±50	293 ±41
Total ω3	153 ±8	200 ±43	207 ±29	193 ±7	251 ±80	220 ±40
Total ω6	66 ±3	576 ±506	1330 ±655	67 ±22	143 ±63	73 ±12

Other includes
12:0,i14:0,14:1,i15:0,a15:0,15:0,16PUFA,i16:0,16:1ω9,16:2,16:1ω5,i17:0,17:1ω8/a17:0,17:0,
18:3ω6,18:4ω3,18:3ω3,18:2,18:1ω5,20:3ω6,20:4ω3,20:2ω6,20:1ω11,20:1ω7,20:0,21PUFA,22:5ω6,
22:4ω6,22:3ω3,22:1ω9,22:1ω7,22:0,23PUFA,24PUFA,24:1,24:0

Table 17.6. Fatty acid composition of cooked Dogfish and cooking oils

mg/100g	Dogfish (Fresh)	Dogfish (Deep fry)	% Dogfish (Fresh)	Dogfish (Deep fry)	Grilling Oil	Deep Fry Oil
14:0	9 ±5	29 ±6	0.4 ±0.1	0.6 ±0.1	0.0	0.6
16:1ω7	56 ±40	85 ±14	2.4 ±0.1	1.7 ±0.1	0.2	0.5
16:0	371 ±246	947 ±148	16.4 ±0.7	18.9 ±1.0	4.3	22.1
18:2ω6	27 ±20	1253 ±266	1.1 ±0.1	25.2 ±5.0	23.6	53.3
18:1ω9	463 ±384	1025 ±202	18.6 ±3.0	20.3 ±1.0	62.1	17.8
18:1ω7	114 ±96	157 ±56	4.5 ±0.8	3.1 ±0.8	3.3	1.0
18:0	82 ±41	165 ±28	4.0 ±1.0	3.3 ±0.2	2.1	2.8
20:4ω6	90 ±51	98 ±27	4.2 ±0.6	1.9 ±0.3	0.0	0.0
20:5ω3	71 ±41	97 ±27	3.3 ±0.4	1.9 ±0.3	0.0	0.0
20:1ω9	124 ±124	141 ±70	4.5 ±1.8	2.7 ±1.2	1.4	0.2
22:6ω3	484 ±238	523 ±111	23.5 ±5.5	10.4 ±0.5	0.0	0.0
22:5ω3	79 ±35	88 ±26	3.9 ±1.1	1.7 ±0.2	0.0	0.0
22:1ω11	15 ±15	20 ±8	0.6 ±0.2	0.4 ±0.1	0.0	0.0
other	324	407	11.9	7.7	2.9	
Total mg/100g	2308 ±1608	5035 ±897				
Total SFA	484 ±307	1180 ±187	21.8 ±1.8	23.5 ±1.3	7.4	26.0
Total MUFA	841 ±715	1510 ±367	33.6 ±6.2	29.8 ±3.8	69.0	19.5
Total PUFA	841 ±444	2187 ±410	40.0 ±7.6	43.6 ±4.5	23.6	54.5
Total ω3	660 ±331	736 ±164	31.8 ±6.9	14.5 ±0.7	0.0	0.0
Total ω6	166 ±104	1406 ±272	7.5 ±0.6	28.2 ±4.6	23.6	53.3

Other includes
12:0,i14:0,14:1,i15:0,a15:0,15:0,16PUFA,i16:0,16:1ω9,16:2,16:1ω5,i17:0,17:1ω8/a 17:0,17:0,18:3ω6,18:4ω3,18:3ω3,18:2,18:1ω5,20:3ω6,20:4ω3,20:2ω6,20:1ω11,20:1ω7, 20:0,21PUFA,22:5ω6,22:4ω6,22:3ω3,22:1ω9,22:1ω7,22:0,23PUFA,24PUFA,24:1,24:0, DIOLS

Table 17.7. Lipid class composition (%) and content (mg/100g) of Mures Gourmet soups

	Prawn bisque	Smokey fish chowder	Le Provencal
mg/100g			
Wax ester	0 ±0	0 ±0	0 ±0
Triglyceride	2227 ±107	1029 ±94	29 ±1
Free fatty	8 ±2	5 ±1	2 ±0
Cholesterol	10 ±0	5 ±0	1 ±0
Phospholipid	25 ±3	54 ±1	23 ±1
% Total lipid	2.3 ±0.1	1.1 ±0.1	0.1 ±0.0
%			
Wax ester	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
Triglyceride	98.1 ±0.1	94.1 ±0.5	53.5 ±1.2
Free fatty	0.4 ±0.1	0.5 ±0.1	2.9 ±0.1
Cholesterol	0.4 ±0.0	0.5 ±0.1	1.8 ±0.1
Phospholipid	1.1 ±0.1	5.0 ±0.3	41.8 ±1.4

Table 17.8. Fatty acid composition of Mures Gourmet soups

mg/100g	Prawn bisque	Smoky fish chowder	Le Provencal	% Prawn bisque	Smoky fish chowder	Le Provencal
14:0	217 ±5	78 ±6	1 ±0	12.0 ±0.3	8.8 ±0.1	2.8 ±0.0
16:1ω7	33 ±2	20 ±1	1 ±0	1.8 ±0.0	2.3 ±0.0	2.6 ±0.1
16:0	674 ±36	237 ±15	6 ±0	37.3 ±0.3	27.0 ±0.1	18.4 ±0.1
18:2ω6	27 ±0	30 ±2	5 ±0	1.5 ±0.1	3.4 ±0.0	13.9 ±0.1
18:1ω9	383 ±20	239 ±15	9 ±0	21.2 ±0.1	27.3 ±0.1	26.1 ±0.2
18:1ω7	15 ±1	13 ±1	1 ±0	0.8 ±0.0	1.5 ±0.0	3.1 ±0.1
18:0	216 ±13	112 ±7	2 ±0	12.0 ±0.2	12.7 ±0.0	5.0 ±0.1
20:4ω6	3 ±0	4 ±0	0 ±0	0.2 ±0.0	0.4 ±0.0	1.0 ±0.0
20:5ω3	6 ±1	6 ±0	1 ±0	0.3 ±0.0	0.7 ±0.0	2.7 ±0.1
20:1ω9	4 ±1	14 ±1	2 ±0	0.2 ±0.0	1.6 ±0.0	5.5 ±0.1
22:6ω3	6 ±0	21 ±2	2 ±0	0.3 ±0.0	2.4 ±0.0	7.0 ±0.1
22:5ω3	4 ±0	4 ±0	0 ±0	0.2 ±0.0	0.4 ±0.0	1.2 ±0.0
22:1w11	1 ±0	5 ±0	0 ±0	0.0 ±0.0	0.6 ±0.0	1.4 ±0.0
other	217	94	3	12.0	10.7	9.2
Total mg/100g	1806 ±81	876 ±59	34 ±0			
Total SFA	1223 ±51	468 ±32	10 ±0	67.7 ±0.2	53.4 ±0.1	28.9 ±0.2
Total MUFA	484 ±26	315 ±21	15 ±0	26.8 ±0.2	36.0 ±0.0	43.2 ±0.3
Total PUFA	99 ±4	93 ±6	10 ±0	5.5 ±0.0	10.6 ±0.0	27.9 ±0.1
Total ω3	18 ±2	33 ±2	4 ±0	1.0 ±0.1	3.7 ±0.0	11.7 ±0.0
Total ω6	34 ±1	36 ±3	5 ±0	1.9 ±0.1	4.1 ±0.0	15.6 ±0.1

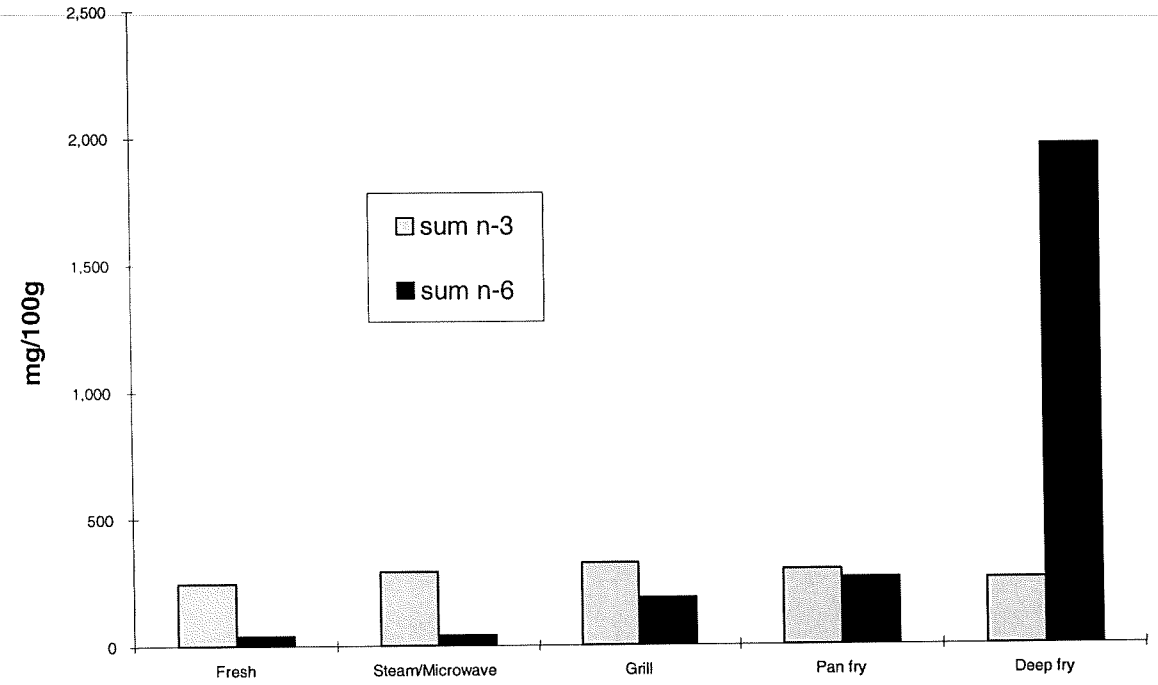


Figure 17.1. Content of n-3 (omega-3) and n-6 LC-PUFA in blue eye, and showing the effect of cooking. Note that results for steaming and microwave cooking were the same.

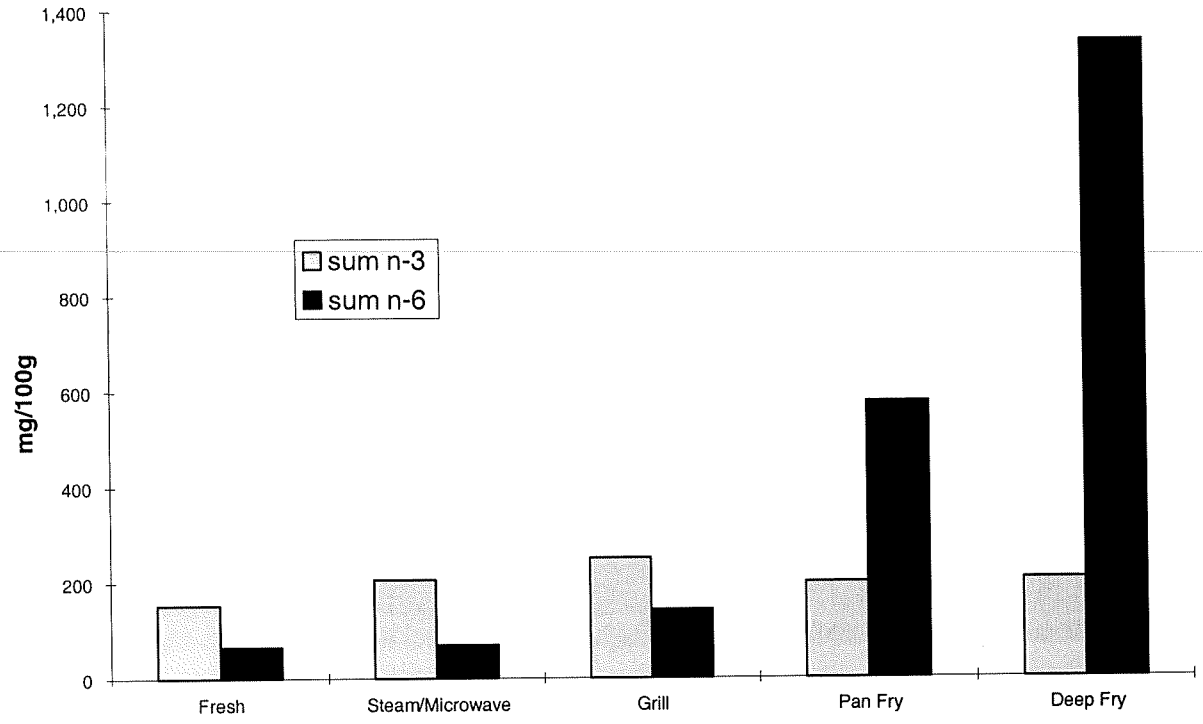


Figure 17.2. Content of n-3 (omega-3) and n-6 LC-PUFA in gummy shark, and showing the effect of cooking. Note that results for steaming and microwave cooking were the same.

18. Analyses of Discoloured Blacklip Abalone

Results in this chapter were provided as a report to the Tasmanian Aquaculture and Fisheries Institute.

Elliott, N., Bartlett, J., Mooney, B. and Nichols, P. (2000) Analyses of discoloured blacklip abalone. Report 2000-TAFI1. Report to Tasmanian Aquaculture and Fisheries Institute.

18.1 Summary

- Identification of possible cause of green discolouration of the foot of the black-lip abalone (*Haliotis rubra*) was requested including possible genetic or oil differences.

- There was no evidence of genetic differences between the two groups of abalone based on analysis at 5 independent microsatellite loci.

- There was no evidence of any differences in oil class, fatty acid nor sterol compositions between the two groups of abalone.

- A UV-visible absorption spectrum analysis produced results consistent with significantly higher concentrations of chlorophyll (160 times greater) occurring in the discoloured foot tissue relative to the white foot tissue.

18.2 Introduction

Members of the Tasmanian abalone industry have reported the presence of discoloured abalone meats in some Tasmanian wild caught blacklip abalone (*Haliotis rubra)*. The extent of the problem prompted one processor to complain that the number of abalone with dark meats was growing, and infer that because the animals were genetically different they should be culled from the stock. Commercial divers report that the discoloured darker individuals are generally found in deep (>12m) waters. Dr Rickard Officer (Senior Research Scientist - Abalone Section, Tasmanian Aquaculture and Fisheries Institute) reported on the international abalone network (ABNET) the presence of discoloured foot tissue. Responses from abalone colleagues suggested that the cause could be either diet or disease related.

As part of FRDC projects 1999/164 (abalone molecular genetics) and 1999/331 (nutritional value of Australian seafood) CSIRO Marine Research undertook to examine samples of the blacklip abalone with normal and discoloured foot tissue from the same location. The examinations were for genetic variation at DNA microsatellite loci, and oil class, fatty acid and sterol compositions.

18.3 Materials and Methods

Fifty-seven frozen blacklip abalone were provided to the CSIRO Marine Research laboratories in Hobart. These abalone were collected on 11 May 2000 from the north-eastern shore of Maria Island, Tasmania. Of these 28 had a normal white foot-muscle, and 29 had a greenish discolouration to the foot tissue. The samples provided were chosen to represent abalone displaying the extremes of light and dark meats from a total catch of over 500kg of blacklip abalone.

Genetic analysis

Standard extraction and purification protocols were used to obtain DNA from all samples. Genetic variation was examined at five microsatellite loci *cmrHr*1.14, *cmrHr*1.24, *cmrHr*2.14, *cmrHr*2.30 (Evans *et al.* 2000) and rubCA1 (Huang and Hanna 1998). Full details of methods including PCR (polymerase chain reaction) conditions for amplification of the microsatellite DNA and gel running conditions on the ABI autosequencer are available from the authors upon request.

The genetic analysis program Genepop (Version 3.2) was used to examine Hardy-Weinberg equilibrium (determines whether a sample is from a randomly mating discreet population and not representative of multiple populations) and genetic differentiation of the two sample groups at each of the 5 loci.

Oil analyses

The foot-muscle from 3 individuals of each of the white and discoloured abalone were analyzed for oil class, fatty acid and sterol composition. Standard analytical procedures developed during FRDC project 95/122 were used; full details are available from the authors

upon request. Oil was extracted from each individual and the extract analyzed using an Iatroscan TLC-FID analyzer to determine the abundance of individual oil classes. Individual fatty acids and sterols were identified using gas chromatography. Chlorophyll levels were recorded for a normal white tissue sample and a discoloured tissue sample by UV-visible absorption spectra between 250 and 700nm using a Shimadzu Variable Absorbance spectrophotometer with a 1cm quartz cell

18.4 Results

Genetic analysis

Only locus (rubCA1) showed disagreement with Hardy-Weinberg equilibrium, after standard correction for multiple tests; this disagreement was for both tissue groups and is therefore likely to be an artifact associated with this locus.

Allele frequencies at all five loci were very similar between the two groups (Table 18.1), and there was no statistical evidence of genetic differentiation at any of the five loci.

Oil analyses

No differences were observed in total oil, oil class (Table 18.2) or fatty acid (Table 18.3) compositions between the normal white coloured and the discoloured individuals.

The major sterol in all samples was cholesterol, ranging from 89-93% (Table 18.4). Other sterols included fucosterol (2-4%), desmosterol (0.4-2%), trans-22-dehydrocholesterol (1-3%) and 24-methylenecholesterol (1-2%). Little difference was observed in the sterol composition of the white and discoloured tissue samples, with most differences attributable to natural variation and to a lesser extent analytical variation.

The UV-Visible absorption spectra for the discoloured sample had a large chlorophyll peak at 665nm, with a corrected absorbance of 0.847. Other peaks were observed at wavelengths of 506nm, 538nm and 608nm. The white tissue sample also had a peak at 665nm, with a corrected absorbance of 0.02. These results are consistent with a higher concentration of chlorophyll (160 times greater) occurring in the discoloured tissue relative to the white tissue.

18.5 Discussion

The analyses conducted indicate no genetic differences between the individual blacklip abalone with discoloured foot tissue and those with normal white foot tissue. The null hypothesis that the abalone all came from the same genetic population cannot be refuted.

The was no difference between the two colour forms of blacklip abalone in terms of oil levels nor composition. However a UV-visible spectrum analysis indicates that the discoloured tissue has higher chlorophyll levels compared with the normal white tissue. There appears to be no related change in individual sterol and fatty acid components, which

has been reported to be associated with dietary differences in abalone (Nelson 1999), to match this finding.

The reason for the high chlorophyll levels is unclear, and the results should be treated with some caution as they come from only two individuals. Nevertheless, it may be speculated that the discoloured individuals were either taking in an unusual form of chlorophyll, their diet was higher in chlorophyll, or their ability to breakdown the chlorophyll was reduced. The absence of any associated differences in the oil composition indicates however, that the diet was similar for the two groups.

Further research is suggested to provide an insight into this phenomenon. Firstly chlorophyll levels in additional samples should be examined, including examination of the effect of depth and location on these. In addition, examination of chlorophyll levels in dietary items and the viscera of discoloured and white individuals would provide an indication of whether the high chlorophyll is from the diet, or due to reduced enzyme activity in particular individuals.

It is concluded from the analyses performed in this study that there is no evidence for any genetic basis to the discolouration of the foot tissue, rather the phenomenon is more likely to be related to the diet of these particular individuals.

Table 18.1. Microsatellite allele frequencies within each sample for five loci. *n* = number of individuals scored for locus.

Allele	White tissue sample	Discoloured tissue sample
Locus *cmrHr*1.14		
1	0.09	0.06
2	0.74	0.78
3	0.06	0.12
4	0.02	0
5	0.02	0
6	0	0.02
7	0.06	0
8	0.01	0.02
n	*27*	*25*
Locus *cmrHr*1.24		
1	0	0.02
2	0.80	0.84
3	0.11	0.09
4	0.09	0.03
5	0	0.02
n	*28*	*29*
Locus *cmrHr*2.14		
1	0	0.02
2	0.02	0.05
3	0.07	0.05
4	0.02	0
5	0	0.04
6	0.38	0.46
7	0	0.02
8	0.39	0.21
9	0.02	0
10	0.11	0.14
n	*28*	*28*

Table 18.1 cont.

Locus *cmrHr*2.30		
1	0.02	0.02
2	0	0.02
3	0.04	0
4	0.02	0.02
5	0.02	0.04
6	0.04	0.09
7	0.04	0.05
8	0.13	0.09
9	0.17	0.02
10	0.02	0.02
11	0.04	0.02
12	0.11	0.07
13	0	0.05
14	0.06	0.05
15	0.04	0.02
16	0	0.02
17	0.06	0.02
18	0.06	0.11
19	0.02	0
20	0.02	0.04
21	0.02	0.02
22	0	0.02
23	0.02	0.02
24	0	0.04
25	0.04	0
26	0.02	0.04
27	0	0.02
28	0.02	0
29	0	0.04
30	0.02	0
31	0	0.02
32	0	0.05
n	*27*	*28*
Locus rubCA1		
1	0.05	0
2	0.21	0.14
3	0.04	0.03
4	0.04	0
5	0.04	0.05
6	0	0.05
7	0.05	0.07
8	0.05	0.02
9	0.02	0.03

Table 18.1 (cont'd)

10	0.04	0.02
11	0.13	0.12
12	0.07	0.07
13	0.04	0.09
14	0.04	0.10
15	0	0.05
16	0.04	0.02
17	0.04	0.03
18	0	0.02
19	0.05	0.03
20	0.04	0
21	0	0.02
22	0.02	0
23	0.02	0
24	0	0.03
n	*28*	*29*

Table 18.2. Percentage total oil and oil class for each of three individuals of white and discoloured foot tissue.

	White tissue samples			Discoloured tissue samples		
Ref. No.	FR828	FR829	FR830	FR831	FR832	FR833
% Total oil (wet wt.)	0.8	0.7	0.8	0.7	0.9	0.8
% Composition of total oil						
Triglyceride	0.0	0.0	0.0	0.0	0.0	0.0
Free fatty acid	0.9	0.7	0.4	0.8	0.4	0.7
Sterol	10.0	9.2	9.7	8.5	8.2	8.5
Phospholipid	89.0	90.1	89.9	90.7	91.4	90.8

Table 18.3. Percentage fatty acid composition for each of three individuals of white and discoloured foot tissue.

	White tissue samples			Discoloured tissue samples		
Fatty acid	FR828	FR829	FR830	FR831	FR832	FR833
14:0	3.5	3.4	2.7	3.2	3.2	3.4
15:0	0.7	0.9	0.9	0.7	0.6	0.8
16:1ω9c	0.5	0.4	0.5	0.4	0.5	0.4
16:1ω7c	2.0	1.9	2.0	1.4	1.6	1.9
16:1ω5c	0.3	0.3	0.3	0.2	0.3	0.3
16:0	18.6	18.7	18.7	21.0	18.7	17.7
i17:0	0.3	0.3	0.2	0.3	0.2	0.2
17:1ω8/a17:0	0.4	0.5	0.5	0.4	0.5	0.4
17:0	1.1	1.4	1.4	1.8	1.4	2.2
18:3ω6	0.6	0.7	0.7	0.5	0.6	0.6
18:2ω6	1.0	0.9	1.0	0.8	1.2	0.7
18:3ω3	0.0	0.0	0.0	0.0	0.0	0.0
18:1ω9c	10.5	11.1	9.6	11.6	11.4	10.2
18:1ω7c	8.2	8.7	8.0	7.8	8.3	8.4
18:1ω5c	0.1	0.1	0.1	0.2	0.3	0.2
18:0	5.7	5.9	5.7	7.2	6.6	5.8
20:4ω6 AA	12.4	11.5	13.8	12.8	13.0	11.7
20:5ω3 EPA	4.1	5.5	3.8	3.1	3.5	3.0
20:3ω6	0.3	0.3	0.3	0.3	0.4	0.2
20:4ω3	0.4	0.3	0.4	0.3	0.5	0.2
20:2ω6	0.3	0.2	0.2	0.2	0.2	0.1
20:1ω11c	2.1	1.7	2.1	1.5	1.8	1.7
20:1ω9c	0.4	0.4	0.4	0.5	0.3	0.3
20:1ω7c	0.2	0.2	0.2	0.2	0.2	0.2
20:0	0.3	0.4	0.3	0.5	0.3	0.3
22:5ω6	0.3	0.1	0.1	0.1	0.1	0.1
22:6ω3 DHA	0.2	0.2	0.2	0.3	0.2	0.1
22:4ω6	2.8	2.6	2.9	3.2	2.7	2.7
22:5ω3	7.2	6.7	7.0	5.9	6.7	5.5
22:2 NMI	3.8	3.9	3.7	3.9	4.1	4.0
22:1ω11	0.2	0.2	0.2	0.2	0.2	0.2
22:0	0.2	0.2	0.2	0.2	0.3	0.2
24:0	0.5	0.1	0.1	0.1	0.0	0.1
other	10.1	9.6	11.0	8.5	9.3	15.4
total	100.0	100.0	100.0	100.0	100.0	100.0
Totals						
Saturates	31.1	31.3	30.5	35.1	31.4	30.7
Monounsaturates	24.9	25.7	23.9	24.3	25.3	24.0
Polyunsaturates	34.1	33.6	34.9	32.2	34.1	29.9
Omega 3s (ω3)	16.4	17.3	15.9	14.4	15.8	13.7
Omega 6s (ω6)	17.7	16.3	19.0	17.8	18.2	16.2

20:2 NMI Coelute with 20:3ω6 and 20:4ω3

Table 18.4. Percentage of individual sterol components for each of three individuals of white and discoloured foot tissue.

	White tissue samples			Discoloured tissue samples		
Sterol	FR828	FR829	FR830	FR831	FR832	FR833
24-norcholesterol	0.3	0.3	0.5	0.3	0.4	0.2
patinosterol	0.5	0.4	0.6	0.3	0.5	0.3
trans-22-dehydrocholesterol	1.4	1.5	3.0	1.2	1.6	1.6
cholesterol	88.9	90.9	89.0	92.6	90.9	90.0
desmosterol	2.2	2.2	1.7	0.7	0.7	0.4
brassicasterol	0.7	0.5	1.0	0.6	1.2	0.7
24-methylenecholesterol	1.9	1.3	1.3	1.3	1.5	2.0
24-methylcholesterol	0.4	0.4	0.6	0.7	0.3	0.2
poriferasterol	0.0	0.1	0.1	0.0	0.0	0.0
fucosterol	3.2	2.4	2.1	1.8	2.2	3.7
isofucosterol	0.5	0.2	0.3	0.1	0.2	0.3
other	0.0	0.0	0.0	0.4	0.5	0.6
Total	100	100	100	100	100	100

19. Oil analyses of abalone as a tool to distinguish the site of origin

Results in this chapter were provided by Nick Elliott as part of a summary report to Mr. Harry Roeding (Natural Resources and Environment, Victoria)

19.1 Introduction

A key issue for the management and conservation of both wild and aquaculture abalone industries is identification. The level or scale at which identification is both warranted or in fact possible is a matter of debate, and depends on the needs for such information. For example, biological data (such as growth data) or more expedient means (such as State boundaries) could provide delineation of management units. However, for compliance purposes the identification of the units needs to be more specific and conclusive. The main focus to date has been on genetic identification, and this is generally recognised as conclusive evidence of identification. When single genetic units exist however, other areas with potential for separation or identification of units include signature lipids of the flesh, and the microchemistry of the shell, both of which reflect the environmental conditions experienced by the relatively sedentary animals.

19.2 Reults and Discussion - Signature lipid profiles

Genetic diversity, whether between species, stocks or individuals, is a biologically meaningful method for unit identification; it is a reflection of the level of gene flow between individuals at the various levels. CSIRO Marine Research has been actively researching the use of genetics in abalone identification on three levels – species, stocks and individuals.

The lipid or oil profile of an individual is a reflection of the individual's diet, i.e. 'we are what we eat'. In theory, if individual abalone are grazing on different algal communities that have different lipid compositions this should be reflected within the lipids of the abalone.

As part of FRDC project 1999/331,we conducted a pilot study on the lipid profiles of abalone from four Tasmanian sites. Fatty acid and sterol profiles for blacklip abalone from each site are shown in Tables 19.1 and 19.2. Hierachial cluster results show promising separation of abalone from the sites sites based on the signature lipid profiles (fatty acids and sterols) of individual abalone (Figure 19.1). In general individuals from a particular site clustered together, more so when using fatty acids, and in particular those from the east site were markedly different to those from the other three sites. These findings are preliminary, and are based on a small number of individuals per site and a small number of sites. In addition, while all animals were of commercial size, no allowances for possible size, sex or seasonal differences have been examined to date.

The results, however, do show promise and suggest that further research utilising signature lipids may be beneficial in identifying management units of abalone based on the unique algal community of particular coastal regions.

Future research in this area should analyse and compare the signature lipids profiles of at least 15 individuals from each of: 1. the disputed catch, 2. the suggested catch location and 3. the suspected catch location (total 45 individuals). Smaller sample sizes could be examined with reduced processing time and costs (can be negotiated). However, for statistical power a minimum comparison of 15 individuals is recommended. Small samples of foot tissue in a frozen state would be required for the analyses.

Table 19.1. Fatty acid composition (%) for black lip abalone from four Tasmanian sites

	North west	North east	South	East
14:0	3.8 ±0.6	3.7 ±0.2	3.3 ±0.2	3.0 ±0.1
i15:0	0.1 ±0.0	0.1 ±0.0	0.1 ±0.0	0.1 ±0.0
a15:0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
15:0	0.7 ±0.1	0.9 ±0.1	1.1 ±0.1	1.5 ±0.1
i16:0	0.1 ±0.0	0.1 ±0.0	0.1 ±0.0	0.1 ±0.0
16:1w9	0.5 ±0.1	0.5 ±0.0	0.6 ±0.1	0.4 ±0.1
16:1w7	1.5 ±0.4	1.7 ±0.3	1.7 ±0.2	1.7 ±0.1
16:1w5	0.1 ±0.0	0.2 ±0.0	0.1 ±0.0	0.1 ±0.1
16:0	18.8 ±0.8	19.9 ±0.8	19.8 ±0.6	21.4 ±0.9
i17:0	0.3 ±0.1	0.4 ±0.2	0.3 ±0.1	0.3 ±0.1
17:1w8/a17:0	0.5 ±0.1	0.5 ±0.0	0.6 ±0.1	0.7 ±0.0
17:0	0.9 ±0.4	0.9 ±0.4	1.5 ±0.1	1.8 ±0.1
18:4w3	0.6 ±0.1	0.7 ±0.1	0.7 ±0.2	0.7 ±0.0
18:2w6	1.2 ±0.1	1.1 ±0.1	1.2 ±0.1	1.0 ±0.1
18:1w9	10.0 ±0.6	8.5 ±0.7	8.5 ±0.5	8.7 ±1.4
18:1w7	8.8 ±0.5	8.8 ±0.6	8.4 ±0.2	7.8 ±0.1
18:1w5	0.1 ±0.0	0.1 ±0.0	0.1 ±0.0	0.1 ±0.0
18:0	5.8 ±0.2	5.4 ±0.4	5.7 ±0.3	5.9 ±0.1
20:4w6	13.9 ±0.9	14.2 ±0.7	15.5 ±0.5	10.4 ±0.0
20:5w3	5.1 ±0.4	5.6 ±0.4	4.8 ±0.7	8.1 ±0.2
20:3w6	0.4 ±0.0	0.5 ±0.1	0.5 ±0.1	0.4 ±0.1
20:4w3	0.4 ±0.0	0.4 ±0.0	0.4 ±0.0	0.3 ±0.1
20:2w6	0.2 ±0.1	0.3 ±0.2	0.2 ±0.0	0.1 ±0.0
20:1w11	0.1 ±0.1	0.1 ±0.1	0.0 ±0.0	0.0 ±0.0
20:1w9	2.7 ±0.4	3.0 ±0.2	2.8 ±0.3	2.3 ±0.3
20:1w7	0.1 ±0.0	0.1 ±0.0	0.1 ±0.0	0.1 ±0.0
20:0	0.0 ±0.0	0.1 ±0.0	0.1 ±0.0	0.1 ±0.0
C21PUFA	0.5 ±0.2	0.4 ±0.1	0.3 ±0.1	0.4 ±0.0
22:6w3	0.1 ±0.1	0.2 ±0.1	0.4 ±0.1	0.2 ±0.1
22:4w6	3.6 ±0.5	3.5 ±0.6	4.2 ±0.4	2.5 ±0.3
22:5w3	11.1 ±1.0	11.3 ±1.0	10.6 ±0.4	12.9 ±0.8
22:2NMI	7.2 ±0.6	5.8 ±0.6	5.3 ±0.4	5.8 ±0.3
22:1w11	0.1 ±0.1	0.1 ±0.0	0.1 ±0.0	0.1 ±0.0
22:1w9	0.4 ±0.1	0.3 ±0.1	0.3 ±0.1	0.3 ±0.0
22:0	0.1 ±0.0	0.1 ±0.0	0.1 ±0.0	0.1 ±0.0
23PUFA	0.2 ±0.1	0.2 ±0.1	0.2 ±0.1	0.4 ±0.0
Sum SAT	30.7 ±1.2	31.6 ±0.8	32.1 ±0.3	34.3 ±0.5
Sum MUFA	25.0 ±0.6	24.0 ±0.9	23.4 ±0.6	22.3 ±0.9
Sum PUFA	44.4 ±1.3	44.4 ±0.6	44.5 ±0.7	43.4 ±1.4
sum n-3	24.5 ±1.3	24.0 ±1.4	22.2 ±0.9	28.2 ±0.8
sum n-6	19.2 ±1.2	19.7 ±1.4	21.7 ±0.8	14.4 ±0.5

20:2 NMI Coelute with 20:3ω6 and 20:4ω3

Table 19.2. Sterol composition for blacklip abalone from four Tasmanian sites

Sterol	North west	North east	South	East
1	0.0 ±0.0	0.3 ±0.0	0.3 ±0.1	0.3 ±0.1
2	0.8 ±0.2	0.8 ±0.1	0.7 ±0.1	0.7 ±0.1
3	0.1 ±0.1	0.3 ±0.1	0.3 ±0.0	0.6 ±0.1
4	1.6 ±0.3	2.2 ±0.2	2.6 ±0.2	2.1 ±0.2
5	88.1 ±1.6	87.7 ±1.5	88.8 ±1.1	87.0 ±0.8
6	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
7	0.7 ±0.4	0.4 ±0.0	0.4 ±0.5	1.8 ±0.1
8	0.6 ±0.2	0.9 ±0.2	0.9 ±0.5	0.0 ±0.0
9	0.2 ±0.1	0.2 ±0.1	0.2 ±0.0	0.2 ±0.0
10	0.4 ±0.1	0.5 ±0.2	0.5 ±0.1	0.4 ±0.1
11	0.6 ±0.1	0.7 ±0.3	0.8 ±0.2	0.9 ±0.1
12	0.6 ±0.1	0.6 ±0.2	0.5 ±0.1	0.5 ±0.0
13	0.3 ±0.1	0.5 ±0.2	0.3 ±0.1	0.3 ±0.0
14	0.4 ±0.2	0.5 ±0.2	0.4 ±0.1	0.4 ±0.1
15	0.8 ±0.1	0.7 ±0.1	0.4 ±0.1	0.5 ±0.0
16	0.8 ±0.1	0.7 ±0.1	0.5 ±0.1	0.6 ±0.1
17	1.1 ±0.2	0.9 ±0.1	0.9 ±0.2	2.5 ±0.5
18	0.3 ±0.1	0.2 ±0.1	0.1 ±0.1	0.0 ±0.0
19	2.0 ±1.0	1.5 ±0.2	0.8 ±0.2	0.4 ±0.0
20	0.9 ±0.2	0.6 ±0.4	0.6 ±0.2	0.7 ±0.1

Sterols include: 1, 24 norcholesterol; 2, cis-22-dehydrocholesterol; 3, cis-22-dehydrocholestanol; 4, trans-22 dehydrocholesterol; 5, cholesterol; 6, cholestanol; 7, desmosterol; 8, brassicasterol. Sterols 9-20 include: 24 methylene cholesterol, stigmasterol, fucosterol, isofucosterol and other components.

Hierarchical Cluster Tree

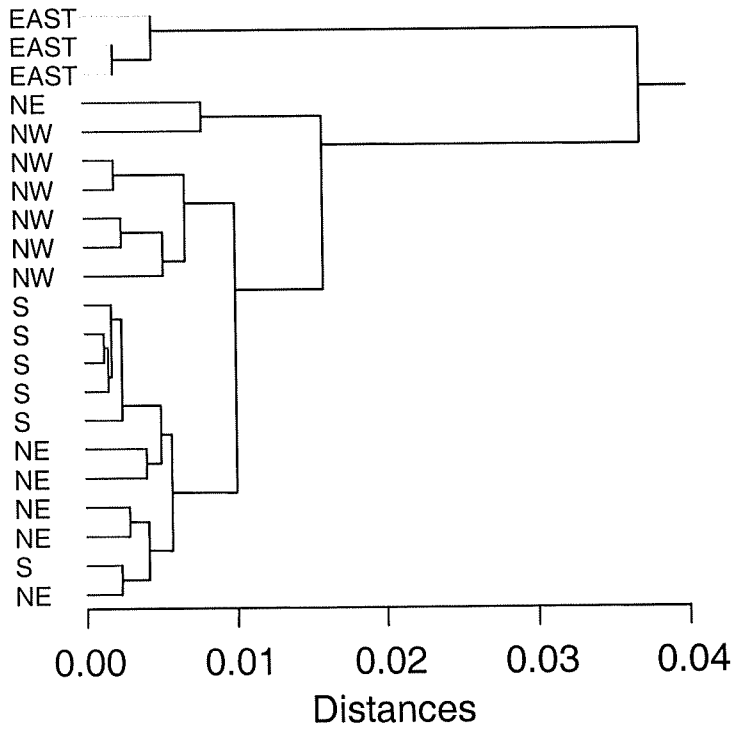


Figure 19.1. Cluster analysis of level of dissimilarity of fatty acid profiles of individual abalone from four sites around Tasmania. NE, northeast; NW, northwest; S, south.

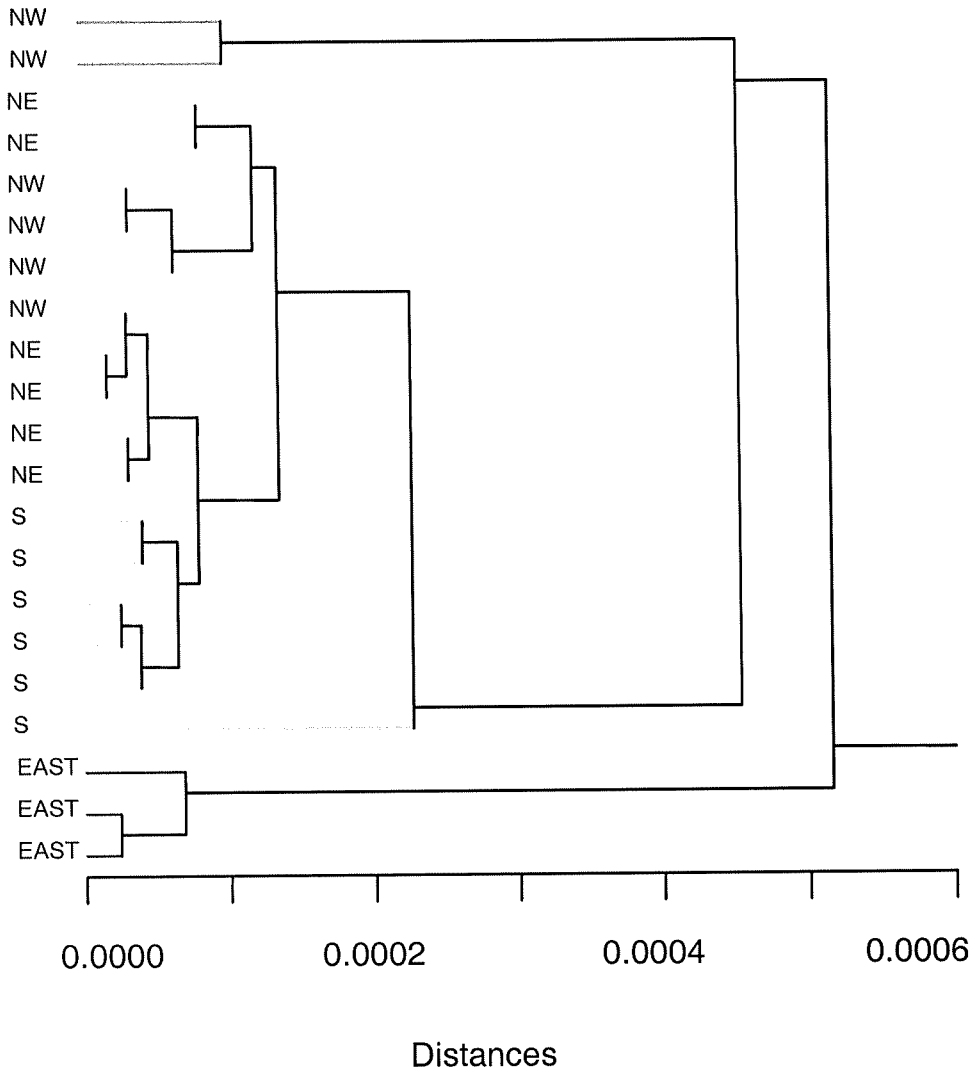


Figure 19.2. Cluster analysis of level of dissimilarity of the signature lipid (sterol) profiles of individual abalone from four sites around Tasmania. NE, northeast; NW, northwest; S, south.

20. A Pilot Study of the Oil Composition of the Eye Orbital of Australian Skipjack Tuna

Results in this chapter were provided as a report to Meo Healthcare.
Nichols, P., Elliott, N and Mooney, B. D. (2000) A pilot study of the oil composition of the eye-orbital of tuna. Internal Report 2000-CMR6.

20.1 Summary

The oil content and composition, and fatty acid composition, were determined for the eye-orbital of skipjack tuna (*Katsuwonus pelamis*). Oil content varied from 2% (eyeball alone, wet weight basis) to 30% (whole eye socket, i.e. including surrounding connecting tissue). The oil yield was 5.4 g oil per eye socket (i.e. 10.8 g oil per tuna head), with the eyeball alone yielding only 0.03 g of oil per eye (0.06 g oil per tuna head). Phospholipid was the major oil class in the eyeball samples, followed by triacylglyceride (TAG). Sterol accounted for 5% of the total oil in the eyeball samples. In comparison, TAG (96%) dominated the oil from the whole eye socket samples. The TAG levels of the whole eye socket oil are considerably greater than observed for tuna oil obtained as a by-product of tuna canning (unpublished data). The major fatty acids in all tuna eye-orbital samples were: 22:6ω3 (docosahexaenoic acid, DHA, 27-30%), 16:0 (palmitic acid), 18:1ω9c (oleic acid), 18:0 (stearic acid) and 20:5ω3 (eicosapentaenoic acid, EPA, 6-10%). The level of DHA compares very favourably with concentrations found in processed tuna oil (DHA, 25%). The oil compositional results for skipjack tuna eye-orbital presented in this report represent to our knowledge the first detailed Australian data for this species. Analyses of further samples of oil from this species may be beneficial to gain insight into compositional variations that may occur due to changes in geographic location, environmental and other factors.

20.2 Results and Discussion

The oil content and composition, and fatty acid composition, were determined for samples of the eye-orbital of skipjack tuna (*Katsuwonus pelamis*). Samples consisted of frozen heads obtained during October 2000 from Port Lincoln. Analytical methods used were thin layer chromatography – flame ionisation detection (TLC-FID) and gas chromatography (GC) / GC-mass spectrometry (GC-MS). Sample work-up and analytical methods were as described in Nichols *et al.* (1994). This study was performed as part of an FRDC-funded investigation (99/331) of the oil composition of Australian seafood.

Two types of samples were examined in either triplicate or quadruplicate:

- Type (i) - eyeball only (eyeball material which was easily removed)
- Type (ii) - whole eye socket (eyeball and surrounding white tissue).

It was possible to remove both types of samples using a laboratory vacuum system (water pump aspiration), however, a more efficient vacuum system would be beneficial. Under the

laboratory conditions used, the surrounding white tissue required minor dissecting for removal.

Oil content

Total oil (lipid) content of the tuna eye-orbital samples varied from 2% to 30% (wet weight basis), with the highest oil content observed for the samples [type (ii)] including the surrounding white tissue (Table 20.1). The samples had been frozen and thawed several times, and some minor discoloration was observed for several samples. The type (ii) samples yielded 5.4 g of oil per eye socket (i.e. 10.8 g oil per tuna head), and the type (i) samples yielded only 0.03 g of oil per eyeball (0.06 g oil per tuna head).

Oil class composition

Phospholipid (PL, 58% +/- 8% of total oil) was the major oil class present in all type (i) samples, followed by triacylglycerol (TAG, 36% +/- 8%). Variation within the type (i) sample replicates was apparent for both TAG and PL. Sterol, mainly cholesterol based on GC analysis, accounted for 5% of the total oil in all type (i) samples.

In comparison, TAG (mean 96%, Table 20.1) dominated in the type (ii) samples. Two replicates contained >97% TAG, with one replicate containing 93.5% TAG. The TAG levels of the oil are markedly greater than observed for tuna oil obtained as a by-product of tuna canning. Solvent extraction was the method used for oil recovery in this study. The solvent extraction method will enhance the recovery of more polar oil classes. It would be anticipated that a conventional heating/separation process would be less effective in recovering the polar oil classes, including coloured materials. Therefore higher levels of TAG, i.e. higher oil quality would be expected using a heating and separation process.

Fatty acid composition

The major fatty acids in all tuna eye orbital samples were: DHA (27-30%), 16:0 (19-20%), 18:1ω9c (13%), 18:0 (3-8%) and EPA (6-10%, Table 20.2). These five components accounted for 72-76% of the total fatty acids in all samples. Polyunsaturated fatty acids (PUFA) were dominant in all samples (44-46%), with lower levels of monounsaturated fatty acids (MUFA, 22-24%) and saturated fatty acids (SFA, 30-34%). Variations in fatty acid composition were apparent between the two types of eye tissue. For example, the type (ii) samples contained slightly lower relative (%) levels of DHA and higher levels of EPA.

Considerable interest exists in the levels of the long-chain (C_{20} and C_{22}) omega-3 polyunsaturated fatty acids (LC-PUFA), in particular EPA and DHA, in seafood and marine oil products. This is the result of well-documented nutritional benefits of these unique PUFA. They help against coronary heart disease, high blood pressure, rheumatoid arthritis, and may also be beneficial against other disorders, including some forms of cancer. The LC-omega-3 PUFA are also important in larval development and survival for many aquaculture species. Most Australian seafood contains elevated levels of the long-chain omega-3 oils,

including the beneficial DHA and EPA (Appendix; Nichols *et al.* 1998a&b). The relative levels of DHA (27%) in the tuna eye orbital (whole eye, type (ii) samples) compares very favourably with concentrations found in processed tuna oil (DHA, 25%; Nichols *et al.* 1998d).

The oil compositional results for skipjack tuna eye orbital presented in this report represent to our knowledge the first detailed Australian data for this species. The oil is attractive in appearance and contains a compositional profile suitable for consideration of further value adding. Analyses of further samples of oil from this species may be beneficial to gain insight into compositional variations that may occur due to changes in geographic location, environmental and other factors.

Table 20.1. Tuna eye orbital - oil composition and content

	(i) Tuna eye ball only n=4	(ii) Tuna whole eye socket n=3
Percent Oil Class		
Wax ester	0.2±0.2	0.0±0.0
Triglyceride	35.8±8.3	96.2±2.1
Free fatty	1.9±1.0	0.5±0.2
Cholesterol	4.7±1.5	0.4±0.2
Phospholipid	57.5±8.0	2.9±1.6
% Total oil (wet weight basis, per eye)	2.0±2.1	30.2±4.7
Mass eye tissue (g) (per head)	3.8	36.2
Oil per eye (g)	0.031	5.4
Oil per head (g)	0.063	10.8

Table 20.2. Tuna eye orbital - fatty acid composition

Fatty Acid	Percentage compostion	
	(i) Tuna eye ball only n=4	(ii) Tuna whole eye socket n=3
14:1	0.1 ±0.0	0.2 ±0.0
14:0	3.5 ±0.8	5.3 ±0.3
a15:0	0.2 ±0.1	0.3 ±0.0
15:0	1.0 ±0.3	1.4 ±0.1
C16PUFA	0.1 ±0.1	0.1 ±0.0
16:1ω9	0.4 ±0.1	0.3 ±0.0
16:1ω7	3.6 ±0.2	5.6 ±0.3
16:2	0.6 ±0.2	0.9 ±0.0
16:1ω5	0.2 ±0.1	0.3 ±0.0
16:0	20.1 ±2.4	18.5 ±0.8
i17:0	0.1 ±0.0	0.1 ±0.0
17:1/a17:0	0.8 ±0.1	1.2 ±0.0
17:0	0.9 ±0.3	1.0 ±0.1
18:3ω6	0.1 ±0.0	0.1 ±0.0
18:4ω3	0.9 ±0.4	0.3 ±0.0
18:2ω6 LA	1.3 ±0.3	2.2 ±0.0
18:1ω9	12.6 ±1.7	13.1 ±0.1
18:1ω7	2.2 ±0.1	2.2 ±0.1
18:2	0.1 ±0.0	0.2 ±0.0
18:1ω5	0.1 ±0.0	0.1 ±0.0
18:0	7.6 ±0.8	2.7 ±0.1
20:4ω6 AA	1.9 ±0.1	1.6 ±0.1
20:5ω3 EPA	6.3 ±0.3	10.2 ±0.1
20:3ω6	0.0 ±0.0	0.1 ±0.0
20:4ω3	0.6 ±0.2	0.6 ±0.0
20:2ω6	0.2 ±0.0	0.2 ±0.0
20:1ω11	0.1 ±0.0	0.1 ±0.1
20:1ω9	0.8 ±0.1	0.6 ±0.0
20:1ω7	0.1 ±0.0	0.1 ±0.0
20:0	0.3 ±0.0	0.2 ±0.0
C21PUFA	0.1 ±0.0	0.3 ±0.0
22:5ω6	0.6 ±0.1	0.8 ±0.0
22:6ω3 DHA	29.8 ±4.8	27.4 ±1.1
22:4ω6	0.2 ±0.0	0.1 ±0.0
22:5ω3 DPA	1.0 ±0.0	1.1 ±0.1
22:1ω11	0.1 ±0.0	0.1 ±0.0
22:1ω9	0.1 ±0.0	0.1 ±0.0
22:1ω7	0.1 ±0.1	0.0 ±0.0
22:0	0.2 ±0.1	0.1 ±0.0
24:1	1.0 ±0.2	0.3 ±0.1
24:0	0.3 ±0.1	0.1 ±0.0
Sum SFA	34.1 ±3.1	29.7 ±1.1
Sum MUFA	22.2 ±2.2	24.2 ±0.4
Sum PUFA	43.7 ±4.1	46.1 ±1.2
sum ω3 PUFA	38.5 ±4.6	39.5 ±1.2
sum ω6 PUFA	4.2 ±0.4	5.1 ±0.2

21. Lipid, Fatty Acid and Sterol Composition of New Zealand Green Lipped Mussel *(Perna canaliculus)* and Tasmanian Blue Mussel *(Mytilus edulis)*

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Abbreviations: NZGLM, New Zealand Green Lipped Mussel; TBM, Tasmanian Blue Mussel; BM, Blue Mussel; NZ, New Zealand; FAME, fatty acid methyl esters; TLC, thin layer chromatography; TLC-FID, thin layer chromatography-flame ionisation detector; GC, gas chromatography; GC-MS, gas chromatography-mass spectrometry; DAGE, diacylglyceryl ether; HC, hydrocarbon; TG, triacylglycerol; FFA, free fatty acid; ST, sterol; PL, phospholipid; 20:5n-3, eicosapentaenoic acid; 22:6n-3, docosahexaenoic acid; DPA, docosapentaenoic acid; MUFA, monounsaturated fatty acid; SFA, saturated fatty acid; PUFA, polyunsaturated fatty acid; n-3, omega 3 PUFA; n-6, omega 6 PUFA; NMI, non-methylene interrupted; 4,8,12-TMTD, 4,8,12-trimethyl tetradecanoic acid; α-OH 16:0, alpha-hydroxy 16:0 fatty acid; i18:0, isomer of 18:0 fatty acid; C16 PUFA, polyunsaturated fatty acid of 16 carbons; SD, standard deviation.

21.1 Abstract

The lipid, fatty acid and sterol composition of the New Zealand Green Lipped Mussel (NZGLM, *Perna canaliculus*) and the Tasmanian Blue Mussel (TBM, *Mytilus edulis*) were compared using TLC-FID and GC-MS. Each mussel species was obtained from three different sites in New Zealand (NZGLM) and Tasmania (TBM), respectively. Lipid class distribution of both mussel species was characterised by a high proportion of phospholipid (PL, 57-79%) and triglyceride (TG, 10-25%), free fatty acids (FFA, 7-12%) and sterols (ST, 12-18%). The NZGLM had higher proportions of TG, FFA and ST ($p<0.01$), whereas the TBM had a higher proportion of PL ($p<0.01$). There were higher proportions of total polyunsaturated fatty acid (PUFA), saturated fatty acid (SFA), omega-3 fatty acids and hydroxy and non-methylene-interrupted (NMI) fatty acids ($p<0.05$) in the TBM compared with the NZGLM. The major fatty acids in the NZGLM were 16:0 (15-17%), 20:5n-3 (14-20%) and 22:6n-3 (11-17%). The same fatty acids dominated for the TBM, although, there

were significantly higher proportions of 16:0 (p=0.000) and 22:6 n-3 (p=0.003) and lower proportions of 20:5n-3 (p=0.0072) in the TBM. The novel PUFA, 28:8n-3, was detected in both mussels with higher amounts in the TBM, which probably reflects a greater dietary contribution of dinoflagellates for this species. Cholesterol was the dominant sterol in both mussels. Other major sterols included brassicasterol, 22-methylcholesterol, *trans*-22-dehydrocholesterol and desmosterol. There were significant differences ($p<0.05$) for twelve of the twenty sterols measured between the NZGLM and TBM. Six sterols showed significant site differences for the NZGLM, and ten for the TBM. The differences in the fatty acid and sterol composition between the two species may be due to the diet of the NZGLM being more diatom-derived and the diet of the TBM having a greater dinoflagellate contribution.

21.2 Introduction

Molluscs are divided into seven classes, however, literature on the lipid composition is only available for a select group, one being the bivalvia (Joseph 1982). The New Zealand Green Lipped Mussel, *Perna canaliculus* (not to be confused with the green lipped mussel *Perna viridis* from the region Hong Kong as investigated by Ching *et al.* (2001), Li *et al.* (2002) and Chong *et al.* (2001) is a bivalve native marine mussel from the mollusca family Mytilidae, found in the deep-sea beds in the Hauraki Gulf in New Zealand's North Island waters (Goode and Wilson 1990; Creber 1987). It is distinguishable from other bivalve species due to the presence of a bright green stripe around the posterior ventral margin of the shell and its distinctive green lip which is visible on the inside of the shell. The flesh and ligaments of the New Zealand Green Lipped Mussel (NZGLM) tend to be larger than that of the Tasmanian Blue Mussel (TBM). It possesses a similar shape to the blue mussel *(Mytilus edulis)*, and can exceed 120 mm in size (Creber 1987). The external colour of the TBM can vary from blue, purple or black. It is found off the southern coastal waters spreading from the lower west to the lower east of Australia and in Tasmanian waters. The TBM varies in size up to 130mm, however, they are more commonly found less than 90 mm in size (Yearsley *et al.* 1998).

Lipid comprises around 2% of the wet weight in the blue mussel (Gordon 1982; Nichols *et al.* 1998; Kluytmans *et al.* 1985) of which approximately 75% are structural lipids (Kluytmans *et al.* 1985). Depending on season and/or the lifecycle of the mussel, lipid content and composition may vary. During various stages of the lifecycle, particularly in female mussels, the blue mussel is able to synthesise 16:0 and 18:0 and their derivatives *de novo* (Kluytmans *et al.* 1985). Other biochemical changes in the mussel may result from variability in metabolic activity, location, sex and spawning (Teshima and Kanazawa 1974).

Mytilus edulis and *Perna canaliculus* like many other marine organisms such as crustaceans, molluscs and fish are not only low in dietary cholesterol, but contain cholesterol-lowering phytosterols (Gylling and Miettinen 1999) and abundant omega-3 PUFA, particularly the long chain fatty acids such as 22:6n-3 and 20:5n-3 (Joeseph 1982); this is unlike most terrestrial organisms, which are rich in omega-6 fatty acids (Naughton *et* al. 1986). The omega-3 PUFA from marine sources have been linked with reducing certain risk factors for cardiovascular disease, such as lowering plasma triglycerides (Nestel 2000), reducing potential thrombosis as well as alleviating the symptoms of inflammatory conditions such as

arthritis and skin disorders (James and Cleland 1987; James *et al.* 2000; Lorenz *et* al. 1989; Whitehouse *et al.* 1997; Rainsford and Whitehouse 1980). Literature has not reported any research conducted on defining the similarities and or differences in lipid composition of the New Zealand Green Lipped Mussel and the Tasmanian Blue mussel and to our knowledge this is the first study that compares the lipid composition of both mussels.

21.3 Materials and Methods

New Zealand Green Lipped Mussels were obtained from three sites in New Zealand (South Island, Marlborough Sound, Coromandel, with water temperatures of 14.6°C, 16.5°C and 16°C, respectively) and transported frozen by air to CSIRO Marine Research in Hobart, Tasmania, Australia, and stored at -20°C for three to five days, until analysis. Upon harvest, mussels were chilled and frozen within 24 - 36 hours of collection. Blue mussels were collected from three sites in Tasmania, Australia (Deep Bay 1, Deep Bay 2, Great Oyster Bay, water temperature unknown) and stored at -20°C for three to five days, until analysis. Upon harvest, mussels were chilled and frozen within 24 hours of collection. Both species of mussels were collected in late spring (October). Data relating to food sources and availability, water quality is unknown, however it is known that TBM from Deep Bay were collected during a dinoflagellate bloom. Lipids were extracted overnight from 3 individual animals from each site using a modification of the one–phase chloroform/methanol/water (2:1:0.8, by vol.) Bligh and Dyer extraction (1959). After phase separation by the addition of chloroform and water (final solvent ratio, 1:1:0.9, by vol.), the lipids were recovered in the lower chloroform phase and the total solvent extract was evaporated *in vacuo* at 40°C. Lipid content was determined to a constant weight under a stream of nitrogen gas. Lipid class analyses were conducted immediately and where other analyses were to be performed, samples were stored at -20°C for no more than three days in a known volume of chloroform.

A portion of the lipid extract for each mussel was analysed using an Iatroscan MK V TH10 thin-layer chromatography-flame ionization detector (TLC-FID) analyser (Tokyo, Japan) to determine the amounts of individual lipid classes. Samples were analysed in duplicate using silica gel SIII chromarods (5 μm particle size) (Drummond Scientific Company, PA, USA) and application by 1 μl disposable micropipettes. Separation was achieved using a polar solvent system of hexane/diethyl ether/glacial acetic acid (60:17:0.1, by vol.) to resolve non-polar components including wax ester (WE), triacylglycerols (TG), free fatty acid (FFA) and sterols (ST). A second non-polar solvent system (hexane/diethyl ether (96:4, by vol.) was used for selected samples to resolve non-polar components such as hydrocarbon (HC) from WE and TG from diacylglyceryl ether (DAGE). After development the chromarods were dried at 80°C to evaporate all remaining solvent and analysed immediately minimising adsorption of atmospheric contaminants. The TLC-FID was calibrated using known standards for each lipid class (Phleger *et al.* 2001). Peaks were quantified using an IBM PC using DAPA software (Kalamunda, Western Australia).

An aliquot of the total lipid extract, including internal standards (C19:0 and C23:0) was transmethylated using a mild acid methylation to obtain fatty acid methyl esters (FAME) and fatty aldehydes (Phleger *et al.* 2001). ST were obtained by alkaline saponification of another

aliquot of the lipid extract and were then converted to their corresponding tri-methylsilyl ethers (OTMSi) by the addition of 150µl of BSTFA (N,O-Bis (trimethylsilyl) trifluoroacetamide) and heating overnight at 60°C. Gas Chromatographic (GC) analyses were performed using a Hewlett Packard 5890 GC on HP7673 A Autosampler, a split/splitless injector, with a FID and an HP5 non-polar 50m cross linked methyl (5% phenyl) silicone fused-silica capillary column with an ID of 0.32mm and 0.17µm film thickness (Hewlett Packard). Samples were injected at 50°C and held for 1 minute. The oven temperature was increased at 30°C/min to 150°C, then at 2°C/min to 250°C and 5°C/min to a final temperature of 300°C which was held for 15 minutes. The injector and detector were maintained at 290°C and 310°C respectively and hydrogen was used as the carrier gas. Peak areas were quantified on an IBM compatible computer using Millenium 32 V.3.05.01 software (Waters Corporation, USA).

GC-mass spectrometer analyses were performed on a ThermoQuest GCQ system (Thermoquest, USA) fitted with an on-column injector. The GC was fitted with a capillary column similar to that described above and GC conditions were the same as described above.

Statistical analyses were conducted using Minitab Version 12.0 for Windows. Where mussel groups were compared, a Two Sample T-Test and Confidence Interval were conducted. Where sites were compared within mussel groups, a One-way Analysis of Variance (ANOVA) was used. Data is reported as mean $\pm$ SD (n=9, 3 animals from 3 sites for each species) in both text and tables and $p<0.05$ was considered significantly different.

21.4 Results

Lipid composition

Total lipid on a wet weight basis was 17.9 mg/g (4.4 SD) in the NZGLM and 12.3 mg/g (4.0 SD) in the TBM ($p=0.013$). The predominant lipid class in both the NZGLM and TBM was PL, 60% and 74% (of total lipid), respectively, followed by TG (22% and 13%, respectively), with lower levels of FFA (12% and 7%, respectively) (Table 21.1). Both mussel species had a low percentage of wax esters (< 0.3%). There was a significantly higher percentage of TG ($p=0.038$), FFA ($p=0.016$) and ST ($p=0.0085$) in the NZGLM compared with the TBM, and a significantly higher proportion of PL in the TBM ($p=0.010$). There were no significant differences in the lipid class composition between sites for the TBM. However, there was a significant difference for FFA in the NZGLM between sites ($p=0.042$).

Fatty acids

Fifty individual fatty acids and fatty aldehydes were identified in the mussels (Table 21.2, data in table not in order of elution shown in Figure 21.1). The predominant fatty acids were palmitic acid (16:0), 20:5n-3 and 22:6n-3 in both mussels, with 16:0 in significantly higher proportions in the TBM. There were significantly higher percentages of 14:0, 16:1n-7c, 16:1n-7t, 16:1n-9c, 17:0, 18:1n-7 c, 18:2n-6, 20:1n-7 c, 20:2 NMI, 20:3n-6, 20:4n-6, 20:5n-3, 21:5n-3, 22:0, 22:4n-6, 22:5n-6 and 22:5n-3 in the NZGLM ($p<0.05$). The TBM had significantly higher percentages of 15:0, 16:0, α-OH 16:0, 4,8,12-TMTD, i18:0, 19:1, 20:2n-6, 22:2 NMI, 22:5n-6 and 22:6n-3 ($p<0.05$). The novel very long chain PUFA 28:8n-3 was

detected in both the NZGLM (0.2%) and TBM (0.8%), with significantly higher levels ($p<0.0001$) in the TBM.

Fatty aldehydes (C_{16} and C_{18}) (derived from plasmalogens) accounted for <0.5% to 5% in the NZGLM and TBM, respectively.

There were several significant differences between sites for the percent fatty acid composition in both the NZGLM and TBM. The differences between sites for the NZGLM were between 15:0, 16:1n-7c, 18:3n-6, 18:1n-9c, 20:5n-3, 20:1n-11c, 21:5n-3, 22:5n-6 and 22:6n-3 ($p<0.05$) and between sites for the TBM for 20:3n-6, i18:0, 4,8,12-TMTD and 28:8n-3 ($p<0.05$).

Polyunsaturated fatty acids were the dominant class of fatty acids in both mussels (Table 21.3). SFA were the next most abundant group with a higher proportion in the TBM followed by MUFA. The NZGLM had a significantly higher percentage of MUFA ($p<0.0001$), while the TBM had a significantly higher percentage of SFA, total omega-3 PUFA, fatty aldehydes, NMI fatty acids, α-hydroxy fatty acids and several unidentified PUFA ($p<0.05$), compared with the NZGLM. There were no significant differences found between sites in the proportions of SFA, MUFA and PUFA for either mussel species.

Sterols

Twenty sterols were identified in the TBM and NZGLM (Table 21.4, Figure 21.2). Relative levels of individual sterols ranged from <0.5% to 32% (of total sterols). Cholesterol was the major sterol in both mussels (30% TBM and 29% NZGLM). The other sterols common to both the NZGLM and TBM were brassicasterol, 24-methylcholesterol, trans-22-dehydrocholesterol, desmosterol and isofucosterol. The NZGLM had a significantly higher amount of brassicasterol, 24-nordehydrocholesterol, occelasterol, lathosterol, 24-methylcholesterol, 23,24-dimethylcholesta-5,22E-dien-3β-ol and isofucosterol ($p<0.05$). The TBM had significantly higher amounts of cholestanol, desmosterol, 24-methylenecholesterol, 24-ethylcholesterol ($p<0.05$) and an unidentified sterol (peak 14). Both mussel types contained around 2% of the C_{30} sterol dinosterol, while a second C_{30} sterol, 4,23,24 trimethyl-5α-cholest-7-en-3β-ol, was also present in the NZGLM. In the NZGLM, animals from site 3 (Coromandel) had significantly higher proportions of 23,24-dimethylcholesta-5,22E-dien-3β-ol ($p=0.002$), 24-methylenecholesterol, isofucosterol and 4,23,24-trimethyl-5α-cholest-7en-3β-ol ($p<0.05$). Mussels from site 1 (South Island) had a significantly higher amount of dinosterol ($p=0.047$) and site 2 animals (Marlborough Sound) had a significantly higher amount of 22-trans-dehydrocholesterol ($p=0.015$).

For the TBM, site 1 mussels (Deep Bay 1) had significantly higher amounts of 24-nordehydrocholesterol, 22-*trans*-dehydrocholesterol, brassicasterol, 24-methylenecholesterol, and an unidentified sterol ($p<0.05$). Site 2 animals (Deep Bay 2) had a significantly higher amount of 24-methylcholesterol while Site 3 animals (Great Oyster Bay) had significantly higher amounts of cholesterol, cholestanol, lathosterol and 23,24-dimethylcholesterol ($p<0.05$).

21.5 Discussion

Lipid composition

Lipid contents in the TBM and NZGLM (1.2% to 1.8%) were similar to values reported in a study investigating the lipid composition of marine invertebrates Pacific North West (Gordon 1982). That study found the BM to have a lipid content of 1.8% in comparison to clams (1.5-1.7%), cockles (0.9-1.7%), horse clams (2%) and fresh oysters (2.5-4%), with crustacea such as crab and shrimp at around 1% and 1.5%, respectively. Yearsley *et al.* (1998) and Nichols *et al.* (1997) reported a lipid content in the TBM of 1.7% wet weight. Perry (1977) investigated the lipid content of various mollusca and found the lipid content varied from 1.5% in a female mollusc *Subninella undulata*, to 4% in the limpet *Cellana tranoserica*. The lipid content results for the TBM and the NZGLM in this study are in agreement with earlier studies of the blue mussel and other molluscs. The main lipid class of NZGLM and TBM was PL (up to 74%), which is consistent with other data for molluscs (Yearsley *et al.* 1998; Gordon 1982; Nichols *et al.* (1997); Perry 1977).

Depending on the length of storage, temperature and climatic conditions, marine invertebrates such as molluscs can undergo various changes in their lipid profiles due to various enzymes. Jeong *et al.* (1990) and Jeong (1999) studied the effect of temperature and storage on lipid oxidation and composition of total lipid and lipid fractions of the oyster *Crassostrea gigas* and the giant ezo scallop *Patinopecten yessoensis*. In any marine source with the presence of unsaturated fatty acids, particularly long chain omega-3 PUFA, there is the risk of deterioration of lipid during storage. Both studies showed with increased storage time and decrease in temperature up to 35°C, there was an increase in oxidation by measures of thiobarbituric reactive substances (TBARS), a decrease in total lipid, polar lipids and PUFA in total and polar lipids and an increase in non-polar lipids and FFA. This data suggests the activity of various lipases and lipolytic enzymes responsible for enzymatic hydrolysis. In the study by Jeong *et al.* (1990), after 12 months of frozen storage there was a decrease in PL leading to an accumulation of FFA causing an increase in neutral lipids. Our study reported the presence of significant proportion of FFA in both the NZGLM and TBM. Since these were not present in lipid extracts for mussels which were placed into extraction solvents within 10 minutes of harvesting, it is likely that the FFA represent hydrolytic degradation as reported by others (Jeong *et al.* (1990); Jeong (1999). Perhaps in our study lipid hydrolysis was occurring during storage, which could account for the high levels of FFA in both mussels.

The differences in lipid content and lipid class composition between the NZGLM and TBM may be due to seasonal variation as there are differences in climate between New Zealand and Tasmania. Differences may also reflect the stage of development of the mussel, particularly the gonadal development (Joseph 1982). In addition, the sex of the mussel can alter lipid content and lipid class composition, since female mussels tend to have lower levels of total lipid (Joseph 1982). The dietary intake of the mussels and the availability of food may also reflect the differences seen between species. Perry (1977) reported that hydrocarbons (HC) in molluscs are mainly from environmental pollution such as fuel oil, crude oil or motorboat oil. Uncontaminated scallops were found to have a limited range of naturally occurring HC (Perry 1977). HC profiles characteristic of pollution, such as a lack

of odd-chain dominance and the presence of unresolved complex material and other specific biomarkers, were not observed in our samples. These observations are consistent with the pristine nature of the sampling locations from which all mussels were collected.

Fatty acids

Nearly half of the fatty acids in each mussel species were PUFA, and up to 42% of the fatty acids in both mussels were omega-3 PUFA, whilst approximately 5% were omega-6 PUFA. The predominant n-3 PUFA were 20:5n-3 and 22:6n-3. The NZ mollusc *Mytilus canaliculus* contains a similar fatty acid profile, dominated by the PUFA, 20:5n-3 and 22:6n-3 (Nichols *et al.* (1997). Many marine species of molluscs are rich in 20:5n-3, with lower levels of 22:5n-3 and 22:6n-3. It has been reported that it is unusual for molluscs to contain high amounts of 22:6n-3 as mussels are unable to biosynthesise it from 20:5n-3 (Perry 1977). Other bivalvia species, such as *Ostrea lutaria* and *Crassostrea gigas,* have also been found to contain high levels of 20:5n-3 and 22:6n-3, with the former being the predominant PUFA. In our study, there was a significantly higher amount of 22:6n-3 (14-20%), particularly in the TBM. In contrast to previous studies, the high proportion of 22:6n-3 in the NZGLM and TBM is probably largely reflecting the composition of the planktonic diet. Various algal species are known to contain elevated 22:6n-3, including dinoflagellates, cryptomonads and certain thraustochytrids as well as zooplankton (Volkman *et al.* 1989; Lewis *et al.* 1999).

Mollusc fatty acid profiles usually contain about 30-40% SFA (Joseph 1982; Gordon 1982; Phleger *et al.* 2001), a level which was found in the present study. The SFA profiles were similar for both species and also to profiles for other molluscs. The male and female *Subninella undulata* contained 16:0 and 18:0 as the main SFA, similarly the gastropod *Austrocochlea constricta* and the gastropod Telescopic creeper, contained approximately one quarter of total fatty acids as 16:0 (Perry 1977).

The presence of 28:8n-3, along with the C_{30} sterol dinosterol, indicates a strong dietary influence, in particular for the TBM. The TBM was collected during an algal bloom of the dinoflagellate *Gymnodinium catenatum* that occurs in southern Tasmanian waters. G. *catenatum* has been recently reported to contain 28:8n-3 and cholesterol, as a major sterol (Mansour *et al.* 1999). Van Pelt *et al.* (1999) reported the presence of 28:8n-3 in the dinoflagellate *Crypthecodinium cohnii.* This dietary link is further supported by the presence of 24-Methyl-5α-cholest-7en3β-ol in the TBM. This sterol is also found in *G. catenatum* (Hallegraeff *et al.* 1991). The higher amounts of 28:8n-3 in the TBM than in the NZGLM, suggests a greater dietary dinoflagellate contribution with this species.

The fatty acid 18:5n-3 has also been used as a marker for dinoflagellate ingestion (Hallegraeff *et al.* 1991) although it is also present in a few other algal species (Volkman *et al.* 1989). The dinoflagellate biomarkers dinosterol and C_{28} PUFA were present in the TBM at higher levels than in the NZGLM, while 18:5n-3 was only a minor component in both mussels and in similar proportions (0.2% to 0.3%).

The anti-inflammatory properties of fish oils are thought to be due to 20:5n-3 and 22:6n-3 (James and Cleland 1997), however Whitehouse *et al.* (1997) have reported that the anti-

inflammatory activity in the NZGLM is associated with PUFA with 4-, 5-, and 6- double bonds. In the present study the total proportion of PUFA with 4-, 5-, and 6- double bonds was 40% in the NZGLM and 43% in the TBM (p=0.92), indicating little difference between these PUFA between species.

Several individual PUFA other than the more commonly reported EPA, DHA and AA have attracted attention due to their perceived health (beneficial) properties, or because they are present as main constituents in only a limited number of species. For the NZGLM, considerable interest exists in the level of eicosatetraenoic acid (ETA, 20:4). Product derived from NZGLM is stated to contain unique ETAs (delta7,11,14,17; delta5,9,12,15; delta5,9,12,16; http://www.aomega.com/ahs/l1116b.htm). This report also stated that the marine lipid complex rich in ETAs from the New Zealand green-lipped mussel *Perna canaliculus* is the most potent of the omega-3 lipids for the prevention of pain associated with inflammation, including arthritis.

Separation of ETAs other than AA (20:4n-6) and 20:4n-3 can be achieved using capillary GC (unpublished data). Using highly sensitive chemical-ionization GC-MS (CI-GC-MS), we sought to determine whether other ETAs were present in the NZGLM and TBM. AA levels in the NZGLM were higher than in the TBM (mean %: 2.4% and 1.7% respectively). Levels of 20:4n-3 were similar in the two mussels. In contrast to other reports (http://www.aomega.com/ahs/l1116b.htm), we could not detect any other ETAs in either mussel. Our detection limits were estimated to be equivalent to between 0.1-0.2% of the total fatty acids. The reason(s) for this apparent absence of these novel fatty acids remains to be determined.

The 20:2 and 22:2 NMI fatty acids were found in similar concentrations (3-5%) to levels occurring in a variety of marine molluscs such as gastropods (Croft, personal communication). Zhukova *et al.* (1986) reported similar proportions of these fatty acids in *Mytilus edulis* (4.6%), the oyster C*rassostrea virginica* (5.8%) and the northern quahog *Mercenaria mercenaria* (2.9%). The presence of these NMI indicates dietary contribution, as they may be derived from algae and zooplankton and result from desaturation of 20:1n-9 and 20:1n-7 (Perry 1977; Zhukova *et al.* 1986; Abad *et al.* 1995). In the study by Abad *et al.* (1995), NMI were present from 2-10%, depending on the season.

The presence of 4,8,12-TMTD has also been previously reported in various molluscs (Croft, personal communication). The American oyster *Crassostrea virginica* contained small amounts of 4,8,12-TMTD and NMI dienoic acids (Zhukova *et al.* 1986). The NMI 20:2 and 22:2 are constituents of red algae, thus their presence may also indicate direct dietary contribution.

Sterols

Compared with other marine invertebrates, molluscs are unique as they contain a wide range of sterols, besides cholesterol (Gordon 1982). Both mussels in the current study contained a similar range of sterols, however, in differing proportions, with cholesterol the most prominent sterol ranging from 27-32%. Another NZ mussel, Mytilus planulatus, has been reported to contain at least 8 of the sterols identified in our mussels (Perry 1977). It may be

difficult to compare sterol profiles due to varying extraction techniques and sensitivity of analytical methods. Sterols in the M. planulatus included trans-22-dehydrocholesterol (10%), cholesterol (46%), desmosterol (6%), 24-methylenecholesterol (10%), 24-methylcholesterol (4%), 24-ethylcholesterol (4%) and stigmasterol (1%). Perry (1977) also reported similar sterol composition in the BM. Plant sterols such as sitosterol and stigmasterol as identified in both mussel species are believed to aid in cholesterol-lowering in humans (Gylling and Miettinen 1999). Cholesterol was the main sterol (46%) followed by brassicasterol (13%), 24-methylcholesterol and 24-ethylcholesterol (both 4%) and trans-22-dehydrocholesterol (2%). Cholesterol was also identified in scallops, up to 60%, (22) with some additional more complex C_{28} and C_{29} sterols.

Gordon (1982) reported desmosterol and brassicasterol in similar amounts in the BM. Desmosterol is thought to be an intermediate in cholesterol biosynthesis. The clam *S. giganteus* and the chiton *L. Japonica* also have the ability to bioconvert 24-methylenecholesterol and 7-cholestenol respectively, but it is not known if sterols other than cholesterol are synthesised by the mussels.

Teshima and Kanazawa (1974) found sterols in the BM and abalone to be predominantly comprised of cholesterol (30% and 93%, respectively), followed by 22-dehydrocholesterol (25% and 6%, respectively) and 24-methylenecholesterol (12% and a trace, respectively). The BM contained desmosterol, β-sitosterol and three unidentified sterols (7%). Gordon (1982) reported cholesterol as the dominant sterol in oysters (50%) followed by brassicasterol/desmosterol (25%) and 24-methylenecholesterol (25%). Teshima and Kanazawa (1974) showed that molluscs have the ability to synthesize cholesterol, however this ability is dependent on the mussel's lifecycle, sexual maturation and sex.

In conclusion, this study provides data for the whole tissue of the NZGLM about which considerable interest exists due to the reported bioactivity in the oil from this species (Whitehouse *et al.* 1997; Rainsford and Whitehouse 1980). The NZGLM had similar lipid content and lipid class composition to the TBM. Differences observed between the two species were probably due to dietary differences and also may reflect seasonal variation, the development of the mussel, particularly the gonads and/or the sex of the mussel which can alter the lipid content and lipid class composition.

21.6 Acknowledgement

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Table 21.1

Lipid class composition (%) and total lipid content for the New Zealand Green Lipped Mussel (NZGLM) and the Tasmanian Blue Mussel (TBM).

	Percent composition[1]								
	NZGLM				TBM				
	Site 1	Site 2	Site 3	Mean	Site 1	Site 2	Site 3	Mean	P value between species
Wax ester	n.d	0.4 ± 0.6	n.d	0.1 ± 0.4	0.5 ± 0.9	n.d	n.d	0.2 ± 0.1	-
Triglyceride	25.3 ± 8.9	17.8 ± 5.1	22.2 ± 5.0	21.8 ± 6.6[2]	17.2 ± 15.7	12.7 ± 3.1	10.5 ± 2.9	13.5 ± 8.6	P=0.038
Free fatty acid	10.7 ± 1.6	14.9 ± 2.4[4]	9.6 ± 2.1	11.7 ± 3.0[2]	9.7 ± 6.4	7.0 ± 1.7	5.4 ± 0.7	7.3 ± 3.8	P=0.016
Sterol	6.8 ± 1.2	5.5 ± 0.3	6.6 ± 0.5	6.3 ± 0.9[2]	4.9 ± 1.2	4.9 ± 1.2	5.1 ± 0.7	5.0 ± 0.9	P=0.008
Phospholipid	57.1 ± 7.8	61.4 ± 5.4	61.7 ± 3.5	60.1 5.5	67.1 ± 20.8	75.1 ± 4.2	78.7 ± 4.1	73.6 ± 12.0[3]	P=0.010
Total lipid (mg/g wet weight)	19.7 ± 5.4	19.1 ± 4.2	14.9 ± 3.4	17.9 ± 4.4[2]	16.0 ± 5.3	10.6 ± 1.7	10.3 ± 1.3	12.3 ± 4.0	P=0.013

n.d, not-detectable

[1] Data for each species as determined by Iatroscan, are the mean of 9 individual animals, representing 3 animals from each of 3 sites (values are expressed as mean ± SD).

[2] Significantly higher than the TBM based on paired T-tests ($p<0.05$).

[3] Significantly higher than the NZGLM based on paired T-tests ($p<0.05$).

[4] Site significantly different from other sites (NZGLM) based on ANOVA ($p<0.05$).

Table 21.2

Fatty acid composition (%) for the New Zealand Green Lipped Mussel (NZGLM) and the Tasmanian Blue Mussel (TBM).

	Percent composition[1]								
	NZGLM				TBM				
	Site 1	Site 2	Site 3	Mean	Site 1	Site 2	Site 3	Mean	P value between species
14:0	3.7 ± 1.0	3.5 ± 1.0	3.2 ± 0.4	3.5 ± 0.8[2]	1.5 ± 1.1	1.4 ± 0.4	1.1 ± 0.1	1.3 ± 0.6	P=0.0000
4,8,12 TMTD[7]	0.5 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	1.3 ± 0.4	1.3 ± 0.1[5]	1.0 ± 0.4[3]	P=0.0110
15:0	0.5 ± 0.1	0.6 ± 0.1	0.7 ± 0.0[4]	0.1 ± 0.03	0.5 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1[3]	P=0.0002
16:1 n-7c[8]	8.6 ± 0.6	7.4 ± 0.1	8.6 ± 0.6[4]	8.2 ± 0.7[2]	4.2 ± 3.7	3.2 ± 0.8	2.5 ± 0.5	3.3 ± 2.0	P=0.0001
16:1 n-7t/16:2	0.7 ± 0.1	0.8 ± 0.2	0.9 ± 0.1	0.8 ± 0.1[2]	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	P=0.0000
16:1 n-9c	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.0[2]	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	P=0.0330
16:0	16.3 ± 0.7	16.8 ± 0.4	16.5 ± 0.1	16.5 ± 0.5	20.1± 0.7	20.1 ± 2.1	20.1 ± 0.4	20.6 ± 1.1[3]	P=0.0000
i17:0	0.5 ± 0.2	0.6 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.7 ± 0.3	0.6 ± 0.2	0.7 ± 0.1	0.7 ± 0.2	P=0.0950
17:0	1.2 ± 0.1	1.0 ± 0.1	1.2 ± 0.1	1.1 ± 0.1[2]	0.8 ± 0.3	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.2	P=0.0000
i18:0	0.5 ± 0.2	0.6 ± 0.1	0.4 ± 0.0	0.5 ± 0.1	0.6 ± 0.2	0.9 ± 0.1	1.1 ± 0.1[5]	0.9 ± 0.3[3]	P=0.0014
18:0	4.4 ± 1.0	4.6 ± 0.5	3.9 ± 0.3	4.3 ± 0.6	3.7 ± 1.5	4.5 ± 0.8	5.1 ± 0.3	4.4 ± 1.1	P=0.7900
18:1 n-9c/18:3	1.9 ± 0.2	2.2 ± 0.1	2.2 ± 0.1[4]	2.1 ± 0.2	2.7 ± 1.4	1.5 ± 0.2	1.5 ± 0.1	1.9 ± 0.9	P=0.4800
18:1 n-7c	3.1 ± 0.1	3.3 ± 0.2	3.2 ± 0.2	3.2 ± 0.2[2]	1.9 ± 0.0	2.1 ± 0.2	2.0 ± 0.3	2.0 ± 0.2	P=0.0000
18:1 n-7t	0.5 ± 0.0	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.04[2]	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	P=0.0000
18:2 n-6	1.4 ± 0.1	1.6 ± 0.1	1.6 ± 0.2	1.5 ± 0.1[2]	1.3 ± 0.3	1.0 ± 0.1	1.0 ± 0.1	1.1 ± 0.2	P=0.0001
18:3 n-6	0.1 ± 0.1	n.d	0.2 ± 0.0[4]	0.1 ± 0.1	n.d	1.8 ± 3.2	n.d	0.6 ± 1.8	P=0.4200
18:4 n-3	1.7 ± 0.4	2.1 ± 0.1	1.7 ± 0.2	1.8 ± 0.3	2.4 ± 1.3	1.4 ± 0.3	1.2 ± 0.1	1.6 ± 0.9	P=0.5500
18:5 n-3	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.2	0.2 ± 0.1	0.3 ± 0.3	P=0.3600
α-OH 16:0[7]	0.4 ± 0.0	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.8 ± 0.2	0.7 ± 0.1	0.7 ± 0.2[3]	P=0.0007
19:1	0.0 ± 0.1	0.1 ± 0.1	0.03 ± 0.1	0.1 ± 0.1	0.3 ± 0.2	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1[3]	P=0.0440
20:2 n-6	0.3 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.7 ± 0.3	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.1[3]	P=0.0006
20:2 NMI[7]	1.0 ± 0.3	1.0 ± 0.2	1.1 ± 0.2	1.1 ± 0.2[2]	0.5 ± 0.2	0.3 ± 0.0	0.3 ± 0.1	0.7 ± 0.3	P=0.0450
20:4 n-6	2.3 ± 0.5	2.1 ± 0.4	2.7 ± 0.3	2.4 ± 0.5[2]	1.5 ± 0.7	1.9 ± 0.2	1.8 ± 0.2	1.7 ± 0.4	P=0.0060
20:5 n-3	20.0 ± 1.0[4]	19.1 ± 0.2	14.9 ± 0.9	17.9 ± 2.4[2]	13.3 ± 3.5	15.1 ± 0.9	15.4 ± 0.8	14.6 ± 2.1	P=0.0072

Table 21.2 (cont'd)

	Percent composition[1]								
	NZGLM				TBM				
	Site 1	Site 2	Site 3	Mean	Site 1	Site 2	Site 3	Mean	P value between species
20:3 n-6	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.5 ± 0.0[2]	0.5 ± 0.1[5]	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	P=0.0026
20:4n-3/20:2 NMI[7]	1.7 ± 0.6	2.5 ± 0.5	2.0 ± 0.3	2.1 ± 0.5	2.5 ± 0.8	2.0 ± 0.4	1.8 ± 0.4	2.1 ± 0.6	P=0.9700
20:1 n-11c	0.6 ± 0.4	3.5 ± 0.4[4]	2.4 ± 1.6	2.2 ± 1.5	2.9 ± 1.7	1.6 ± 1.7	2.5 ± 1.4	2.4 ± 1.5	P=0.8300
20:1 n-9c	2.2 ± 0.6	0.0 ± 0.0	0.8 ± 1.4	1.0 ± 1.3	1.1 ± 1.9	1.7 ± 1.5	0.9 ± 1.5	1.2 ± 1.5	P=0.7200
20:1 n-7c	1.7 ± 0.4	1.8 ± 0.3	1.1 ± 0.1[4]	1.5 ± 0.4[2]	0.8 ± 0.1	0.9 ± 0.1	0.8 ± 0.2	0.8 ± 0.1	P=0.0004
21:5n-3	0.5 ± 0.1[4]	0.4 ± 0.1	0.4 ± 0.1	0.9 ± 0.2[2]	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.4 ± 0.1	P=0.0000
22:5 n-6	0.3 ± 0.1	0.2 ± 0.1	0.4 ± 0.1[4]	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.1	1.1 ± 0.1[3]	P=0.0120
22:6 n-3	12.5 ± 0.4	12.3 ± 0.9	15.8 ± 1.3[4]	13.5 ± 1.9	18.2 ± 5.5	21.3 ± 2.0	24.2 ± 0.8	21.2 ± 3.9[3]	P=0.0003
22:4 n-6	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1[2]	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	P=0.0000
22:5 n-3	1.5 ± 0.3	1.6 ± 0.2	1.3 ± 0.1	1.5 ± 0.2	1.1 ± 0.2	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	P=0.0007
22:2 NMI[7]	1.0 ± 0.3	1.0 ± 0.2	1.1 ± 0.2	2.1 ± 0.4	1.4 ± 0.4	1.4 ± 0.2	1.2 ± 0.3	2.6 ± 0.5[3]	P=0.0320
22:0	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.04[2]	0.0 ± 0.1	0.0 ± 0.1	n.d	n.d	P=0.0032
C23 PUFA	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.0	0.2 ± 0.1	P=0.0360
28:8 n-3	0.4 ± 0.3	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.2	0.6 ± 0.1	0.9 ± 0.1	0.9 ± 0.2[5]	0.8 ± 0.2[3]	P=0.0000
Other[6]	0.7 ± 1.1	0.4 ± 0.3	0.7 ± 0.5	3.1 ± 1.7	1.5 ± 0.7	0.8 ± 1.0	0.7 ± 0.6	5.0 ± 2.6	P=0.0870

n.d, not-detectable

[1] Data for each species are the mean of 9 individual animals, representing 3 animals from each of 3 sites (values are expressed as mean ± SD).

[2] Significantly higher than the TBM based on paired T-tests ($p<0.05$).

[3] Significantly higher than the NZGLM based on paired T-tests ($p<0.05$).

[4] Site significantly different to other sites (NZGLM) based on ANOVA ($p<0.05$).

[5] Site significantly different to other sites (TBM) based on ANOVA ($p<0.05$).

[6] Other (includes 17:2 and C_{22} PUFA, Dimethylacetal (DMA) of 16:0 and 18:0 fatty aldehyde).

[7] 4,8,12 TMTD, 4,8,12-trimethyl tetradecanoic acid; NMI, Non-methylene interrupted, α-OH 16:0, Alpha hydroxy 16:0 fatty acid.

[8] 16:1w7t is clearly separated from 16:1w13t on the HP5 capillary column

Table 21.3

Fatty acid composition (%) for the New Zealand Green Lipped Mussel (NZGLM) and the Tasmanian Blue Mussel (TBM).

	Percent composition[1]								
	NZGLM				TBM				
	Site 1	Site 2	Site 3	Mean	Site 1	Site 2	Site 3	Mean	P value between species
Saturated fatty acids	27.8 ± 1.2	28.6 ± 0.7	27.6 ± 0.2	28.0 ± 0.9	28.9 ± 1.2	29.5 ± 2.7	30.4 ± 0.6	29.6 ± 1.6[3]	P=0.023
Polyunsaturated fatty acids	44.6 ± 0.4	44.5 ± 0.6	43.7 ± 2.3	44.2 ± 1.3	44.0 ± 7.4	49.2 ± 4.6	50.1 ± 1.2	47.8 ± 5.2	P=0.086
Monounsaturated fatty acids	18.2 ± 0.8	18.2 ± 0.8	18.4 ± 0.5[2]	18.2 ± 0.6	11.9 ± 4.4	10.4 ± 1.0	9.6 ± 0.8	10.6 ± 2.5	P=0.000
Total n-3 fatty acids	37.5 ± 0.4	37.8 ± 0.6	36.1 ± 2.2	37.1 ± 1.4	38.2 ± 7.2	41.7 ± 1.8	44.6 ± 1.4	41.5 ± 4.7[3]	P=0.025
Total n-6 fatty acids	5.1 ± 0.4	5.2 ± 0.4	6.0 ± 0.5	5.4 ± 0.6	4.6 ± 0.4	6.1 ± 3.0	4.3 ± 0.2	5.0 ± 1.8	P=0.550
Other	7.5 ± 2.2	6.5 ± 0.5	8.1 ±1.9	7.4 ± 1.6	12.4 ± 2.8	9.5 ± 2.8	8.4 ± 2.3	10.1± 2.9[3]	P=0.030

[1] Data for each species are the mean of 9 individual animals, representing 3 animals from each of 3 sites (values are expressed as mean ± SD).
[2] Significantly higher than the TBM based on paired T-tests ($p<0.05$).
[3] Significantly higher than the NZGLM based on paired T-tests ($p<0.05$).
[4] Other includes hydroxy, isobranched, NMI fatty acids, fatty aldehydes and unidentified PUFA.

Table 21.4
Sterol composition (%) for the New Zealand Green Lipped Mussel (NZGLM) and the Tasmanian Blue Mussel (TBM).

Sterol	Percent composition[1]								
	NZGLM				TBM				
	Peak number	Site 1	Site 2	Site 3	Mean	Site 1	Site 2	Site 3	Mean	P value between species
24-Nordehydrocholesterol	1	3.5 ± 0.2	4.2 ± 0.4	3.7 ± 0.1	3.8 ± 0.4[3]	3.4 ± 0.1[6]	2.1 ± 0.4	2.1 ± 0.3	2.5 ± 0.7	P=0.0040
Occelasterol	2	4.0 ± 1.3	3.2 ± 1.6	2.8 ± 0.5	3.3 ± 1.2[3]	1.7 ± 0.2	1.5 ± 0.2	1.3 ± 0.2	1.5 ± 0.2	P=0.0019
Trans-22-dehydrocholesterol	3	10.6 ± 0.3	14.0 ± 2.1[5]	10.0 ± 0.3	11.5 ± 2.2	12.0 ± 2.2[6]	8.6 ± 0.8	8.7 ± 0.7	9.8 ± 2.0	-
Cholesterol	4	28.4 ± 1.2	31.9 ± 5.6	27.7 ± 1.1	29.3 ± 3.5	26.6 ± 1.3	31.2 ± 1.0	32.1 ± 1.8[6]	30.0 ± 2.7	-
Cholestanol	5	0.6 ± 0.1	0.9 ± 0.2	0.8 ± 0.1	0.8 ± 0.2	1.6 ± 0.3	2.9 ± 0.4	3.5 ± 0.5[6]	2.7 ± 0.9[4]	P=0.0003
Desmosterol	6	8.2 ± 0.4	8.5 ± 2.5	7.6 ± 0.5	8.1 ± 1.3	10.8 ± 2.4	10.3 ± 1.3	8.9 ± 0.5	10.0 ± 1.5[4]	P=0.0140
Brassicasterol	7	14.7 ± 0.4	14.2 ± 2.0	14.7 ± 0.4	14.6 ± 1.1[3]	11.0 ± 0.5[6]	9.1 ± 0.4	8.6 ± 0.7	9.6 ± 1.1	P=0.0000
Lathosterol	8	0.9 ± 0.6	1.0 ± 1.1	0.7 ± 0.2	0.9 ± 0.6[3]	0.1 ± 0.1	0.3 ± 0.0	0.5 ± 0.1[6]	0.3 ± 0.2	P=0.0320
Lophenol/Ergosterol	9	n.d	n.d	n.d	n.d	2.0 ± 0.5	1.4 ± 0.3	1.3 ± 1.2	1.5 ± 0.4	-
24-Methylenecholesterol	10	n.d	n.d	1.6 ± 0.3[5]	0.5 ± 0.8	6.6 ± 0.7[6]	4.4 ± 0.8	4.7 ± 0.4	5.2 ± 1.1[4]	P=0.0000
24-Methylcholesterol	11	16.7 ± 0.6	13.6 ± 6.8	10.3 ± 0.5	13.6 ± 4.4[3]	6.0 ± 1.2	11.7 ± 0.1[6]	11.2 ± 1.0	9.6 ± 2.7	P=0.0420
23,24-Dimethylcholesta-5,22E-dien-3β-ol	12	2.7 ± 0.3	1.8 ± 1.0	4.8 ± 0.1[5]	3.1 ± 1.5[3]	1.4 ± 0.2	1.9 ± 0.1	2.1 ± 0.1[6]	1.8 ± 0.3	P=0.0320
Porifasterol	13	n.d	n.d	n.d	Tr[2]	n.d	n.d	n.d	Tr[2]	-
Unidentified	14	1.3 ± 0.2	1.4 ± 0.8	1.5 ± 0.0	1.4 ± 0.4	3.1 ± 0.4	3.5 ± 0.6	3.2 ± 0.3	3.3 ± 0.4[4]	P=0.0000
24-Ethylcholesterol	15	n.d	n.d	0.2 ± 0.3	0.1 ± 0.2	1.5 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	1.3 ± 0.1[4]	P=0.0000
Isofucosterol	16	3.9 ± 0.3	2.3 ± 1.3	10.8 ± 1.1[5]	5.7 ± 4.0[3]	2.7 ± 0.6	2.4 ± 0.2	2.5 ± 0.2	2.5 ± 0.3	P=0.0480
Dinosterol	17	3.6 ± 0.5[5]	2.7 ± 1.7	1.0 ± 0.2	2.4 ± 1.5	1.9 ± 0.3	1.9 ± 0.5	2.2 ± 0.3	2.0 ± 0.3	-
Unidentified	18	n.d	n.d	n.d	n.d	2.4 ± 0.2	1.9 ± 0.5	2.0 ± 0.1	2.1 ± 0.3	-
4,23,24-Trimethyl-5α-cholest-7-en-3β-ol	19	n.d	n.d	1.6 ± 0.2[5]	0.5 ± 0.8	n.d	n.d	n.d	n.d	-
Unidentified	20	n.d	n.d	n.d	n.d	0.9 ± 0.4[6]	n.d	n.d	0.3 ± 0.5	-

[1] Data for each species are the mean of 9 individual animals, representing 3 animals from each of 3 sites (values are expressed as mean ± SD). [2] Trace, less than 0.1%, n.d, not detectable. [3] Significantly higher than the TBM based on paired T-tests ($p<0.05$). [4] Significantly higher than the NZGLM based on paired T-tests ($p<0.05$). [5] Site significantly different to other sites (NZGLM) based on ANOVA ($p<0.05$). [6] Site significantly different to other sites (TBM) based on ANOVA ($p<0.05$).

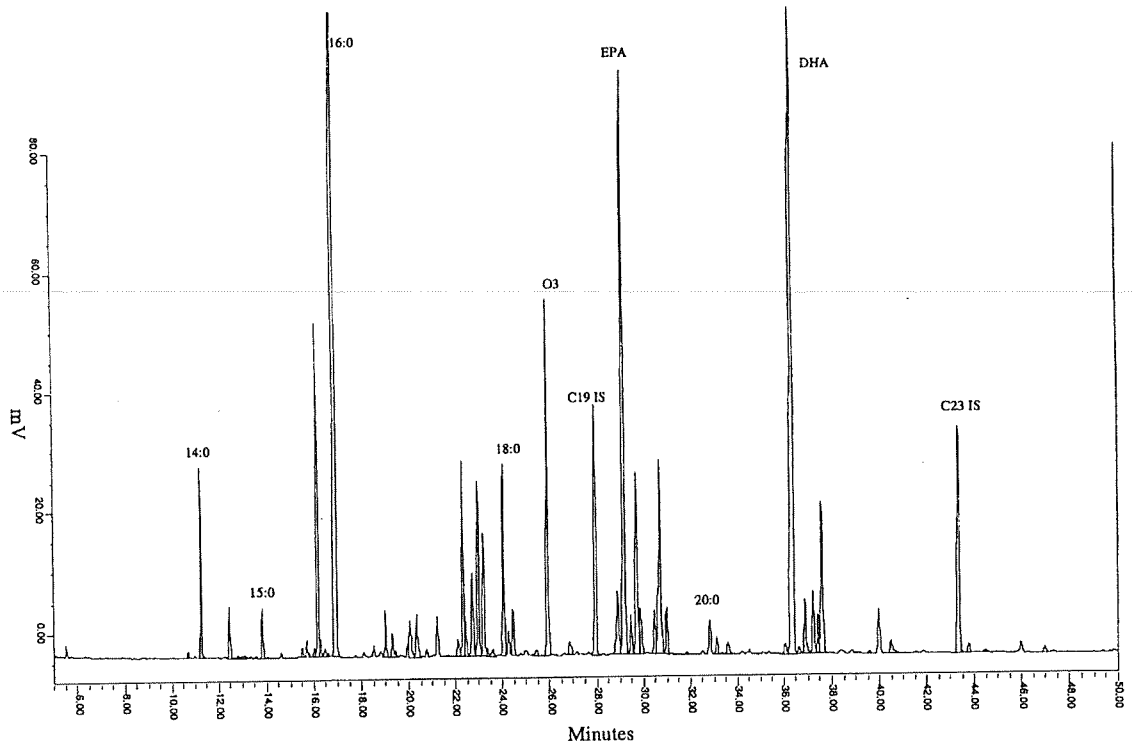

Figure 21.1 Representative fatty acid methyl ester profile of Tasmanian Blue Mussel. Separated on a HP5 (non-polar) 50m cross-linked methyl (5% phenyl) silicone fused-silica capillary column, using a Hewlett Packard 5890 GC with FID. Abbreviations refer to Table 21.2.

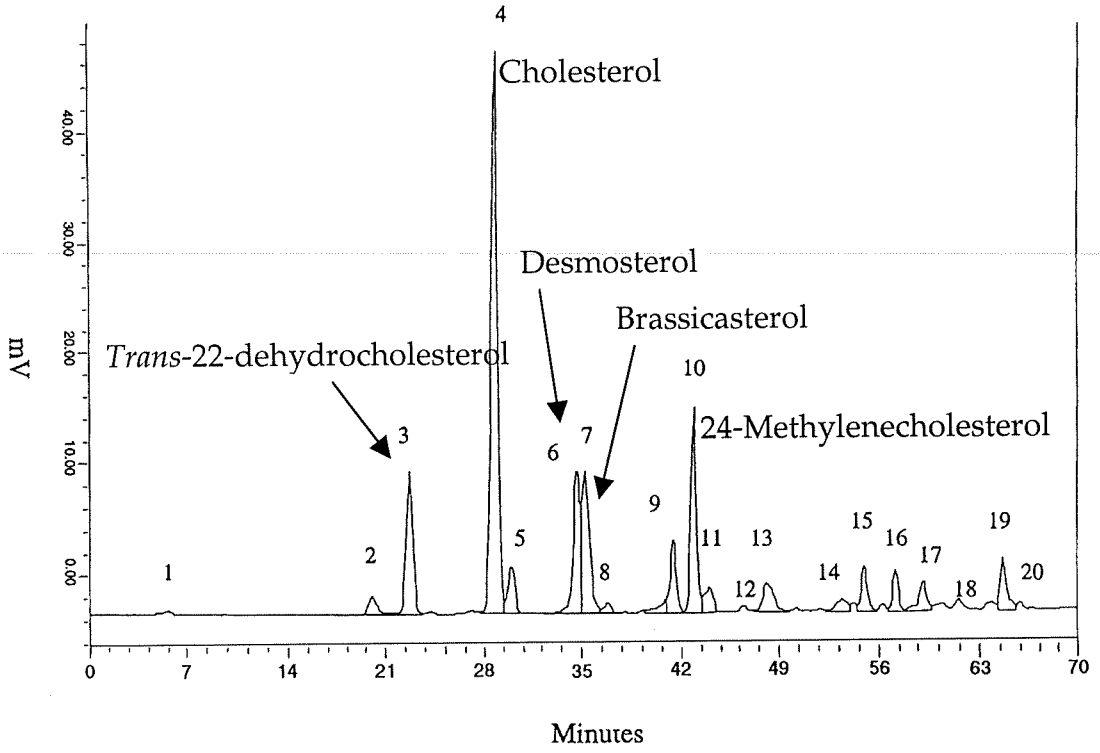

Figure 21.2 Representative sterol profile of New Zealand Green Lipped Mussel. Separated on a HP5 (non-polar) 50m cross-linked methyl (5% phenyl) silicone fused-silica capillary column, using a Hewlett Packard 5890 GC with FID. Peak numbers refer to Table 21.4.

22. Value-adding to Australian Marine Oils

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22.1 Abstract

Marine biotechnology in Australia is a relatively new field. Australia has one of the largest exclusive economic zones (EEZ) globally, an area known to be rich in marine biodiversity from polar, temperate and tropical waters. It has been acknowledged for some time that although Australia is surrounded by a rich marine resource, we are not taking advantage of the potential benefits from the abundant biodiversity. Future research efforts will address this shortcoming. An overview on aspects of current Australian research and development in marine biotechnology, focusing on marine omega-3 oils, will be presented. A comprehensive data-base has been established on the nutritional composition (oils emphasis) of principal Australian seafood. Results are available for strategic marketing of Australian fish species as well as for use by medical and consumer groups. Most Australian fish contain high levels of the nutritionally important omega-3 polyunsaturated fatty acids (PUFA) - eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3). Australian fish generally have higher relative levels of DHA compared to fish and oils from northern hemisphere waters. Tuna are a particularly good source of DHA-rich oil, with the manufacture of Australian-made, value-added tuna oil products commenced in 1998. The tuna oil products contain in excess of 25% DHA, a DHA to EPA ratio of >3, and a lower proportion of cholesterol than is found in flesh from tuna and some other seafood. Species specific differences and regional variation in lipid and fatty acid profiles of highlight the ecological significance of this class of biochemical compounds; in addition changes in oil profiles with cooking and aquaculture are examined. Microbial sources of PUFA have also been developed, with a range of applications possible. Lipid class profiles of wax ester rich oil fishes enabled a species associated with consumer illness to be identified.

22.2 Introduction

Early Commonwealth Scientific and Industrial Research Organisation (CSIRO) research in the 1930s suggested that the oil and vitamin content of a range of shallow and mid water Australian fish had potential to be value-added (Jowett and Davies 1938). After 1950, the availability of synthetically produced vitamins saw the use of fish-derived oils diminish. Limited research and development then occurred in Australia, with purified omega-3 polyunsaturated fatty acid (PUFA) containing oils being imported over the past few decades. However, crude omega-3 PUFA fish oils were being exported or used in lower value products such as feeds for the aquaculture industry.

There is increasing nutritional interest in the omega-3 long-chain (C_{20} and C_{22}) polyunsaturated fatty acids (LC-PUFA). In particular, eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3) in seafood and marine oil products receive special attention. This is the result of the well-documented nutritional benefits of these unique PUFA (Howe 1998; Howe and Nestel 1992; Kinsella 1986 and 1987; Simpoulos 1989). They help against coronary heart disease, high blood pressure, rheumatoid arthritis, and may also be beneficial against other disorders, including some forms of cancer, depression and other neural illnesses. Australian research over the past decades on omega-3 PUFA oils has included

characterisation of these oils from a range of edible seafood species (Belling *et al.* 1997; Evans *et al.* 1986; Fogerty *et al.* 1986; Gibson 1983; Mann *et al.* 1995; Nichols *et al.* 1998 a,b,c; Sinclair *et al.* 1983).

Development of new or refined processing conditions suitable for use with Australian fish oils has occurred, with transfer of know-how and technology to industry through research and licensing agreements. Uses for the omega-3 PUFA oils include health and nutritional products, infant formula and, in the case of lower value material, aquaculture and other feeds. More recent marine oils research has focused on specific nutritional requirements of new aquaculture species. Research is also performed on shark liver and wax ester rich oils, with new Australian products reaching the national and international markets during the 1990s (e.g. nutraceuticals, degreasers, hand cleaners, cutting oils) (Nichols *et al.* 1994, 1997) 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.

In this report, we highlight our research on characterizing the oils of Australian seafood, examining the effects of processing and aquaculture on the oil content and composition of fillets, demonstrate commercial uptake of the research, and describe novel sources of oils and uses of oil profiles in ecological studies.

22.3 Materials and Methods

Samples

Seafood, by-product and oil samples were provided by CSIRO colleagues, industry collaborators and others. Unless otherwise stated, samples analysed were consistently taken from the right shoulder region of fish, and the tail of crustaceans. Effects of processing or cooking involved sampling the same region of individual fillets before and after treatment. Results presented are generally the mean of three samples. All samples were stored frozen prior to analysis. Microbial samples referred to were prepared as described in (Lewis 2001; Lewis *et al.* 1998a,b; Nichols *et al.* 1998 e)

Oil analyses

Full details on oil (lipid) analyses are provided elsewhere (Nichols *et al.* 1998 a,c,e). Briefly, samples were extracted using a single phase Bligh and Dyer procedure (Bligh and Dyer 1959). Oil yield was determined gravimetrically. An aliquot of the oil was analysed by TLC-FID to determine lipid class composition. Fatty acid profiles were obtained by capillary GC and GC-MS analysis following transmethylation of an aliquot of the extracted oil.

22.4 Results and Discussion

Oil composition of Australian seafood

A comprehensive database has been established on the content and composition of the oils from nearly 300 species of Australian fish, shellfish and crustaceans (Nichols *et al.* 1998 a & b, 2002). Results have been published in the Guide *Seafood the Good Food I and II* (Nichols *et al.* 1998; Mooney *et al.* 2002) , and are available for marketing of Australian fish and for use by various medical, nutritional, consumer and research groups. The data also has been incorporated within nutritional data-bases.

For the Australian species analysed during our study, summary findings were:

- Local fish had lower levels of oil than species from northern hemisphere waters
- Most Australian fish have high levels of omega-3 PUFA and low levels of cholesterol.

- Australian seafood contain attractive levels of omega-3 PUFA, typically tenfold or more greater than other food groups (Table 22.1). The key omega-3 PUFA are EPA and DHA. Representative results are provided in Figure 22.1.
- The relative level of PUFA in fish (expressed as % of total fatty acids) generally increased with decreasing oil content, suggesting that better oil quality occurs for the low oil species, and that Australian fish are an excellent source of the essential omega-3 PUFA
- Prawns (shrimps) have lower levels of omega-3 PUFA and higher levels of cholesterol compared with fish
- Australian fish have higher relative levels of DHA compared with fish, and nutritional supplements containing fish oil, from northern hemisphere waters
- fishes from warmer waters and those with specific diets have lower omega-3 / omega -6 ratios compared with fishes from temperate waters, due largely to higher relative levels of arachidonic acid (AA)
- Season, diet and other factors can influence the oil and fatty acid content and composition of seafood.

Our findings, together with results from earlier Australian reports, provide oil compositional information for use in the selection of fish or fish oils for nutritional studies and for the marketing and communication of the health benefits of seafood. These studies indicate that seafood is clearly the best source of omega-3 LC-PUFA from the common food groups (Table 22.1). Standardised procedures were adopted during the study, however, intra-species variation may occur. The results serve as a guide for medical practitioners, nutritionists and other user groups. Comparative nutritional studies with seafood and/or specific marine oils of known oil composition and content will provide additional information on the health benefits of omega-3 LC-PUFA.

Intake of the omega-3 PUFA is well documented to aid in decreasing the incidence of coronary heart disease and stroke in humans and also play a role against a range of other disorders, including arthritis (Howe 1998; Howe and Nestel 1992; Kinsella 1986 and 1987; Simpoulos 1989). Recent nutritional studies have also indicated that DHA may be more active than EPA for certain disorders. Australian seafood, and oils derived from various species, contain generally higher relative levels of DHA and may well prove to be nutritionally more beneficial than those from northern hemisphere waters. Species-specific differences and regional variation in lipid and fatty acid profiles were also observed (Figure 22.1), highlighting the ecological significance of this class of biochemical compounds.

Cooking seafood – effect on oil composition

A frequently asked question is whether different cooking methods affect the nutritional value of seafood. A popular local fish species - blue eye trevalla (*Hyperoglyphe antarctica*) – was chosen to examine the effect of cooking on oil composition. Fresh blue eye contains 1.4% oil, with polar lipid and triglyceride as the main oil classes (84% and 14% respectively, Table 22.2). The omega-3 LC-PUFA, predominantly DHA and EPA, accounted for approximately 35% of the total fatty acids, with total PUFA at 41%. DHA is the dominant component at 27% (Table 22.2). On an absolute basis, the average omega-3 LC-PUFA content was 244 mg/100 g (Figure 22.2), which is slightly higher than the average for Australian fish (210 mg/100 g; see Table 22.1).

Specimens of the blue eye were cooked in five ways: grill, steam, microwave, pan and deep fry. Oil content varied depending on the method of cooking used. A peanut oil was used for deep frying, while a cottonseed based oil was used in the pan frying and grilling. Cooking by microwave or steaming did not affect oil and omega-3 LC-PUFA content and composition relative to fresh fish (Table 22.2, Figure 22.2). Oil content of blue eye increased from 1.4% in fresh fish, to 2.3% with pan frying and 5.3% with deep frying. The higher oil content observed

in the deep-fried and pan-fried fish is consistent with uptake of cooking oil by the fillet during frying. This is also reflected in the oil class results; the deep fried sample contained 84% triglyceride and 14% polar lipid, the complete opposite to the fresh sample at 14% triglyceride and 84% polar lipid. Similarly, pan frying and grilling also increased the triglyceride at the expense of polar lipid. This was not seen with microwaving or steaming.

The content of the main omega-3 LC-PUFA - DHA and EPA - was largely not affected by cooking (Figure 22.2). The differences that were observed in the absolute amount of these beneficial PUFA (e.g. grilling increase to 324 mg/100 g) can be attributed to within-sample variation, and also due to loss of water during cooking.

Examination of the fatty acid profiles expressed in percent form shows that the deep-fried and pan-fried and grilled fish contained higher relative levels of vegetable oil derived components. As noted with the higher oil content observed with these cooking methods, this feature is due to incorporation of cooking oil by the fillet. The deep-fried fish contained higher levels of the omega-6 PUFA linoleic acid (LA, 18:2ω6, 43% cf 1% in fresh samples, Table 22.2) consistent with the use of peanut oil. Oleic acid (OA, 18:1ω9c) was elevated in the pan-fried blue eye (41%). OA is the major fatty acid (62%) in the cottonseed oil used for pan-frying, with LA is dominant (53%) in peanut oil used for deep frying. Steaming and microwaving had no observable effect on oil content and composition in blue eye.

This study represents, to our knowledge, one of the first detailed comparative studies of the effect of various cooking processes on the oil content and composition of an Australian species. Importantly, no loss of the beneficial omega-3 LC-PUFA was observed for any of the forms of cooking examined. An increase in oil content and levels of specific components was seen with frying and grilling, reflecting uptake of vegetable oil components. In terms of nutritional value, whilst the content of omega-3 LC-PUFA did not decrease, the ratio of omega-3 to omega-6 fatty acids did decrease markedly with frying and grilling. Such changes in oil, in particular fatty acid, profiles will need further nutritional evaluation. The use of cooking oils containing lower levels of omega-6 PUFA may be worthy of consideration.

Soup products derived partly from seafood by-products can provide a further source of omega-3 LC-PUFA (Figure 22.2, Table 22.2), although in this instance levels in the soup were lower than observed for the fresh and cooked fish. A variety of seafood by-products can contain high concentrations of the beneficial omega-3 LC-PUFA (e.g. salmon frames and belly flap contain higher levels omega-3 LC-PUFA than occurs in fillet). In this study, only low amounts of omega-3 PUFA were found for the fish-derived soup product (Figure 22.2), therefore scope exists to further increase the level of beneficial oils in soups or stocks.

Farmed fish – the effect on oil composition

Barramundi (*Lates calcarifer*) is a popular wild-caught table species harvested by commercial and recreational fishers from northern Australian waters. Wild-harvested specimens contained on average 0.4% oil (in saltwater specimens) and 0.8% (freshwater specimens) (Nichols *et al.* 1998 a,b) In comparison, cultured barramundi contained 10% oil (wet weight basis), with triglyceride as the main oil class (97%, Table 22.3). In wild-harvested specimens, polar lipid was the dominant oil class. The main fatty acids in cultured barramundi in decreasing order of abundance were: 18:1ω9, 16:0, DHA, 16:1ω7, EPA, 14:0, 18:2ω6 and 18:0; these eight components accounted for 77% of the total fatty acids.

The omega-3 LC-PUFA, predominantly DHA and EPA, accounted for 22% of the total fatty acids in cultured barramundi, with total PUFA at 29%. DHA was the dominant PUFA at 10%. The relative (percent) level of omega-3 LC-PUFA in cultured barramundi was similar to wild-caught freshwater barramundi (26%), but lower than in wild-caught saltwater barramundi (43%).

The ratio omega-3 PUFA / omega-6 PUFA was higher (3.0) in cultured fish compared to the wild fish (0.8-1.9).

On an absolute basis, the omega-3 LC-PUFA content of cultured barramundi was 1970 mg/100 g (Figure 22.3); this content is markedly higher than in most wild-caught seafood. In comparison to wild-caught fish, cultured fish fed marine oil based diets generally contain higher levels of the beneficial oils.

Under the current feeding practices cultured barramundi is an excellent source of the beneficial omega-3 LC-PUFA. Should fish meal and fish oil in current aquaculture diets be replaced with other protein and oil sources, the current high levels of omega-3 LC-PUFA may decrease, as may the product and nutritional value. Scope to further increase the level of beneficial oils in cultured barramundi through manipulation of diet also may exist.

By-product oils

In addition to research devoted to examination of the oil composition of edible species, collaborative research and development has occurred to better utilize marine resources (Nichols *et al.* 1998d)

Tuna oil

Of the Australian fishes, tuna were found to be a particularly good source of DHA-rich oil. Tuna oil is derived as a by-product from canning operations. Collaborative research and development by CSIRO and Clover Corporation, presented an opportunity to exploit the waste derived from processing Pacific tuna. The joint venture company Nu-Mega Lipids commenced manufacture of Australian-made, value-added tuna oil products in early 1998.

The tuna oil products contain around 25% DHA, and a DHA to EPA ratio of >3. It is these two parameters in particular that make the oil suitable, after the addition of AA, for nutraceutical application in infant formulas. When used as a nutritional supplement, consumption of two capsules (2 x 1 g) per day of tuna oil would provide higher levels of DHA (approximately 500 mg, Figure 22.1) than is present in an average serve of Australian seafood (average LC-omega-3 PUFA, 210 mg/100 g; Table 22.1). In comparison, for most Australians the average intake of EPA and DHA is about 100 mg/day, which is less than half that recommended by the UK Department of Health, and only one-tenth of the average population intake recommended by a British Nutrition Foundation Task Force (howe 1998). Of additional interest was the observation that in analyses of purified tuna oil performed to date, we have noted a lower proportion of cholesterol (typically 0.1% or less of the oil) than is found in flesh from tuna and other seafood (average cholesterol in fish, 28 mg/100 g, Figure 22.1, (Nichols *et al.*, 1998b,d).

In comparison to the tuna oil supplement (500 mg DHA per 2 g oil), 2 g of a traditional fish oil supplement (e.g. MaxEPA capsules) contains 240 mg of DHA. Similarly, 2 g of newly developed plant-derived (microalgae) supplements available to the US market provide 400 mg of DHA (Nichols P. and Mooney B., unpublished data, 1997). The tuna oil supplement therefore generally contains higher levels of DHA than other supplements currently available.

Other oils

Liver oil profiles have been obtained for southern and northern Australian sharks, together with by-product oils from other fisheries. Examination of the fatty acid profiles indicates that new sources are potentially available for omega-3 (Figure 22.4) and also diacylglyceryl ether containing oils. These two types of oils have been manufactured over the last 5 years in Australia from by-catch and by-products of other fisheries. The scope may exist to utilize by-products of the northern shark fishery and other fisheries as new scources for these valuable oils.

Infant formula and functional food products

The use of fish oil supplements has gained considerable interest in a variety of applications, including as an additive in infant formulas. It has been demonstrated that with unsupplemented bottle feeding, a deficiency of DHA content (up to 50%) exists in erythrocyte lipid, phosphatidylcholine and phosphatidylethanolamine compared to breast-fed infants (Putman *et al.* 1982; Simopoulos 1989) DHA supplemented (fish oil source) formula-fed infants exhibited a more rapid rate of development of visual acuity compared to control formula-fed babies (Uauy 1990; Uauy *et al.* 1990) The AA : EPA : DHA ratio in Northern Hemisphere fish oil (commonly 0.2 : 2.1 : 1.0 (Singh and Ward 1997) differs markedly from human milk (2 : 0.2 : 1). The high levels of EPA in fish oils from northern hemisphere species may act as an antagonist or an inhibitor of the infant's own endogenous AA synthesis, therefore infant formula may require AA co-supplementation (Singh and Ward 1997).

Flesh from a select few Australian fishes contains the three essential fatty acids at a ratio more similar to that observed in breast milk (Nichols *et al.* 1998b). However, to our knowledge, by-product oils containing the three essential fatty acids at the ratio found in breast milk are not available from Australian fishes. As noted above, tuna oil contains a high DHA to EPA ratio. After microencapsulation using a process developed by Food Science Australia and Clover Corporation, tuna oil is now being added to infant formula with either AA or gamma-linolenic acid (GLA, 18:3ω6) (see www.clovercorp.com.au). Tuna oil is also being utilized in new Australian bread products, and will be incorporated into other functional foods in the future. These examples highlight that the Australian fishing and associated industries do have the capacity to better utilise existing resources.

A perceived issue for the use of single cell oils (SCOs) from microalgae in infant formula is the possibility of toxicity problems. Noteworthy in this 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) The safety of 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). As oil derived from fish generally does not contain unusual components, including e.g. unknown sterols, use of fish oil in infant formula would overcome this potential problem.

Microbial oils

Marine microorganisms contain an array of bioactive molecules, including oils, that have benefits in aquaculture, nutriceuticals, in new pharmaceuticals or as lead compounds. 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. It is evident that certain strains of Antarctic bacteria do produce high levels of PUFA (Nichols *et. al.* 1996) The ability to produce PUFA can allow 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 omega-3 PUFA. PUFA incorporation into live feeds (rotifers) has been demonstrated using both Antarctic bacteria (Lewis *et al.* 1998 a,b; Nichols *et al.* 1996, 1998e) and several of recently isolated novel Australian thraustochytrids (Lewis 2001). The latter group of microheterotrophs represent an attractive source of PUFA-rich oils, with new strains isolated that are rich in DHA and AA (Figure 22.5).

Species identification

Health complaints have occurred recently in Australia associated with the consumption of fillets sold as "rudderfish". The marketing group rudderfish consists of species from three trevalla (family *Centrolophidae*) genera, *Centrolophus*, *Schedophilus* and *Tubbia*, with several undescribed species and uncertain distribution in Australian waters. Consumers had purchased "rudderfish" fillets and, after cooking and eating, some had suffered severe diarrhoeal effects not usually associated with these fish, but reported for others such as escolar (or oil fish) (Yearsley *et al.* 1999). Concern existed over the species identity of the fillets and the cause of the health effects, particularly as both rudderfish and escolar are both caught as long-line by-catch and anedoctal evidence suggested that escolar species were being sold as rudderfish. The name "escolar" in Australian fisheries includes two known gemfish (family *Gempylidae*) species, *Lepidocybium flavobrunneum* and *Ruvettus pretiosus*. The latter species has also been referred to as "castor oil fish (Nevenzal *et al.* 1965) Both species have reported purgative properties (Berman *et. al.* 1981; Yearsley *et al.* 1999)

We compared the oil content and composition profiles, with particular emphasis on the non-saponifiable lipids, of two fish fillets associated with consumer illness, with those obtained for reference samples from the escolar and rudderfish groups. The analyses, supported by general protein fingerprinting (Elliott N., unpublished data, 1999) of the samples, highlight the potential for lipid profiles to be used for identification of selected escolar and rudderfish samples to at least group level.

Both of the unknown fillet samples that had been associated with consumer illness had a very high oil content (>22%, as % wet weight) and the dominant lipid class was wax ester (97%, as % of total oil, Figure 22.6). Such a high oil content and unusual composition were similar to that found in the reference escolar samples and very different to rudderfish specimens (Figure 22.6).

Although the lipid class and fatty alcohol profiles for *L. flavobrunneum* and *R. pretiosus* are very similar, higher levels of 18:1ω9 and 16:1ω9, and lower levels of 18:0 were observed in *R. pretiosus*. Based on comparison of the fatty alcohol profiles, the unknown samples grouped more closely with *L. flavobrunneum* than with *R. pretiosus*. This finding is consistent with results obtained from general protein fingerprinting of the two unknown (Elliott N., unpublished data, 1999) and reference samples (Yearsley *et al.* 1999)

Members of the escolar and rudderfish groups are unusual in containing very high levels of oil (14-25%, as % of total oil) and having oil composition profiles different to most seafood. Oils from seafood, with some exceptions, are generally rich in triacylglycerol and / or polar lipid. The unusual oil profiles of specific members of the escolar and rudderfish groups are not consistent within their families. Members of both families (*Gempylidae* and *Centrolopidae*) are known to have more conventional triacylglycerol or polar lipids.

The first report on the purgative properties of *R. pretiosus* occurred in 1841 (Lowe 1841) More recently, and based on the high wax ester content in Japanese specimens of *L. flavobrunneum* and *R. pretiosus* (Nevenzal *et al.* 1965) the diarrhoea and seborrhoea-producing activity of these fishes was investigated (Mori *et al.* 1966). Based on the results for feeding trials in rats, the flesh and acetone-derived oil of both species were deemed not suitable for human food.

High levels of wax ester rich oil in orange roughy has been reported (fillets 7-10% oil; oil composition 95% wax ester) (Elliott *et al.* 1990; Bakes *et al.* 1995). Extrapolating results obtained from feeding growing rats and pigs with orange roughy, it was proposed that "normal consumption" of orange roughy by humans was unlikely to cause serious health problems (James *et al.* 1986). However, the level of wax ester oil in escolar (18-24%) is nearly three times greater than in orange roughy. Wax ester oils derived from orange roughy and oreo dories have been

incorporated into several industrial cleaning, degreasing and other products in the past decade (Nichols *et al.* 1994 and 1997). Based on the findings of this study, escolar may represent an additional source of wax ester oils for consideration by industry. The results, and the possible incorrect naming of the fillets, suggest that consumers should be made aware of the oil type in these two groups and that strict use be made of recommended marketing names to avoid similar health issues.

22.5 Conclusion

In summary, research and developments with omega-3 and other marine oils have occurred in Australia over the past decade and an exciting era is being entered where the potential benefits from the abundant marine biodiversity will be both increasingly understood and utilised. The Australian Marine Oils industry has progressed rapidly over the past 5 years and opportunities are now available to consolidate developments and commence new initiatives. Basic research opportunities also exist for using oil composition profiles in food-web studies, including as part of ecosystem studies. 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.

22.6 Acknowledgement

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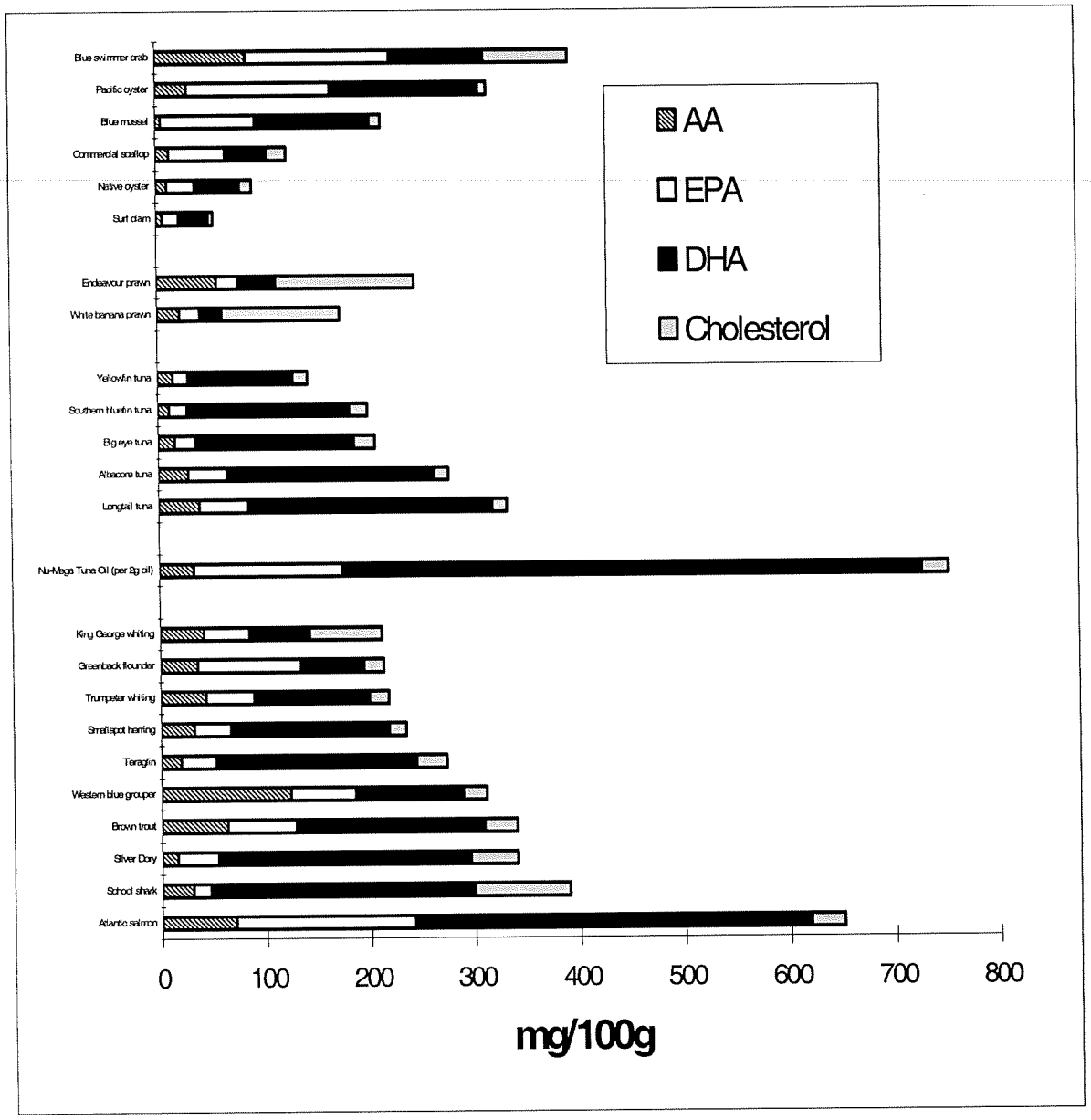

Figure 22.1. AA, EPA, DHA and cholesterol levels of selected Australian seafood (mg per 100 g serving) and for purified tuna oil (mg per two 1 g capsules).

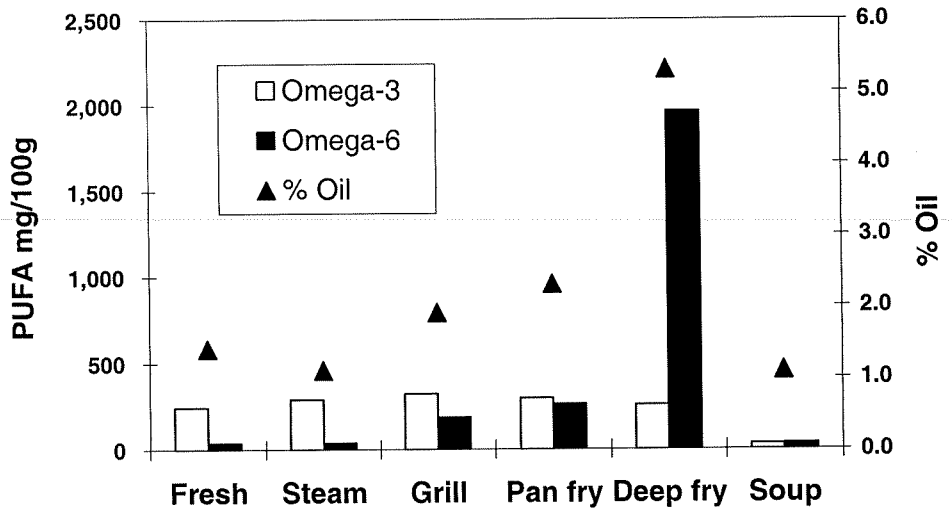

Figure 22.2. The effect of cooking on the content (mg/100 g) of omega-3 PUFA and omega-6 PUFA and oil (%, wet mass) in blue eye trevalla fillets and fish soup.

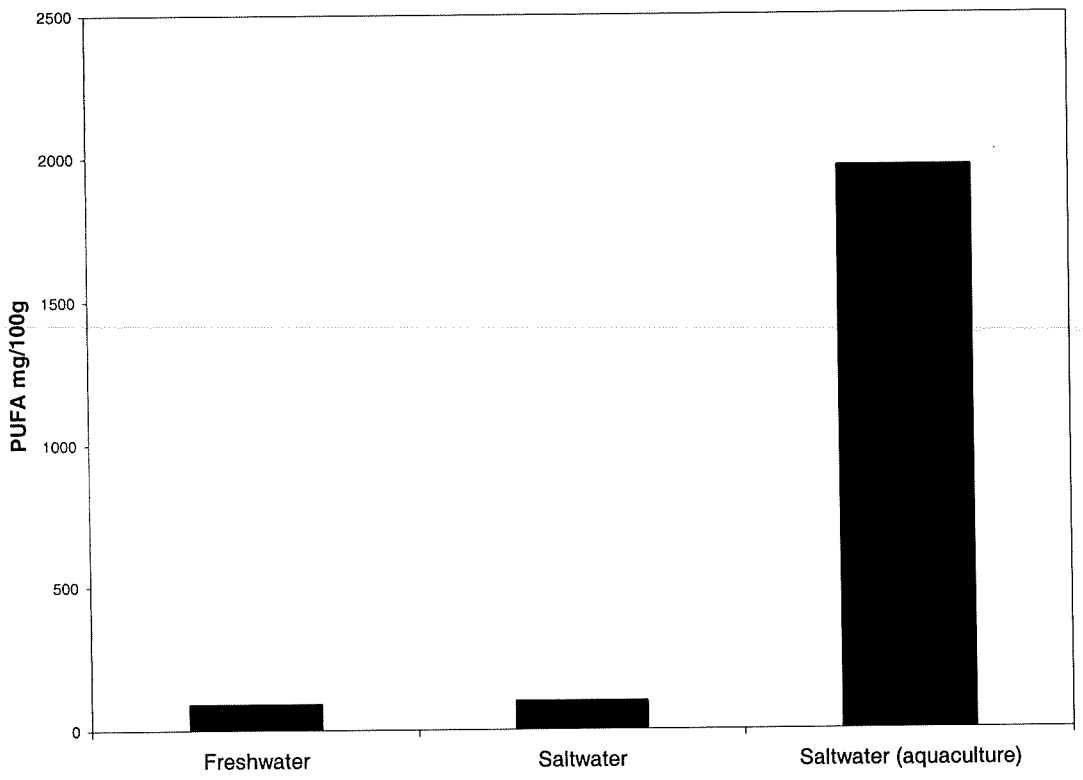

Figure 22.3. Content of omega-3 LC-PUFA in wild-caught (freshwater and saltwater specimens) and cultured barramundi.

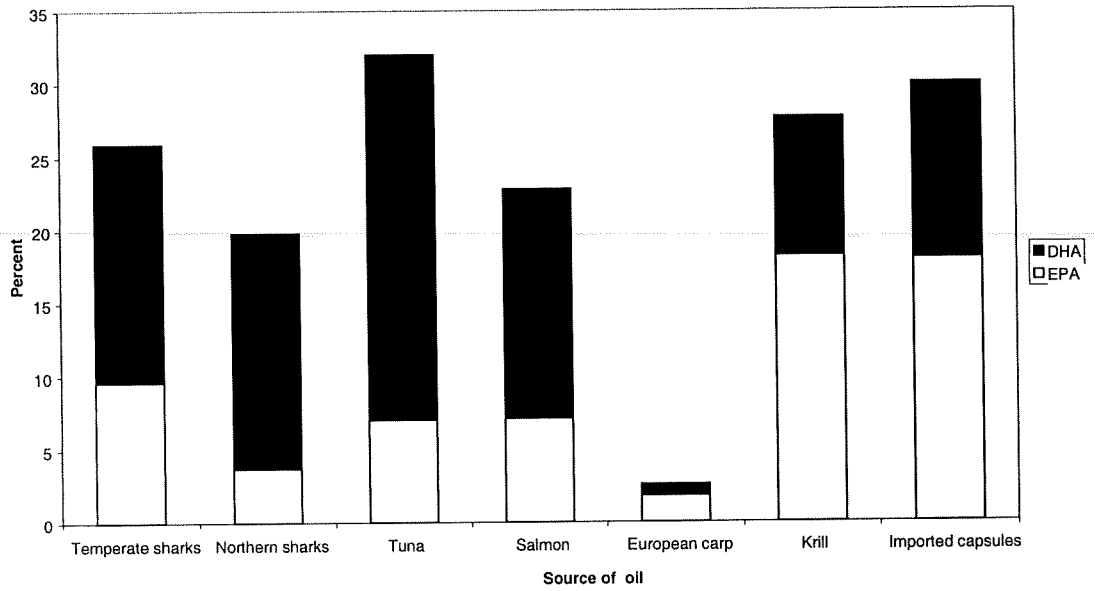

Figure 22.4. EPA and DHA composition (as % of total fatty acids) of selected by-product oils from Australian species compared with that in imported fish oil capsules. Northern shark data represents mean data for 41 species (unpublished data).

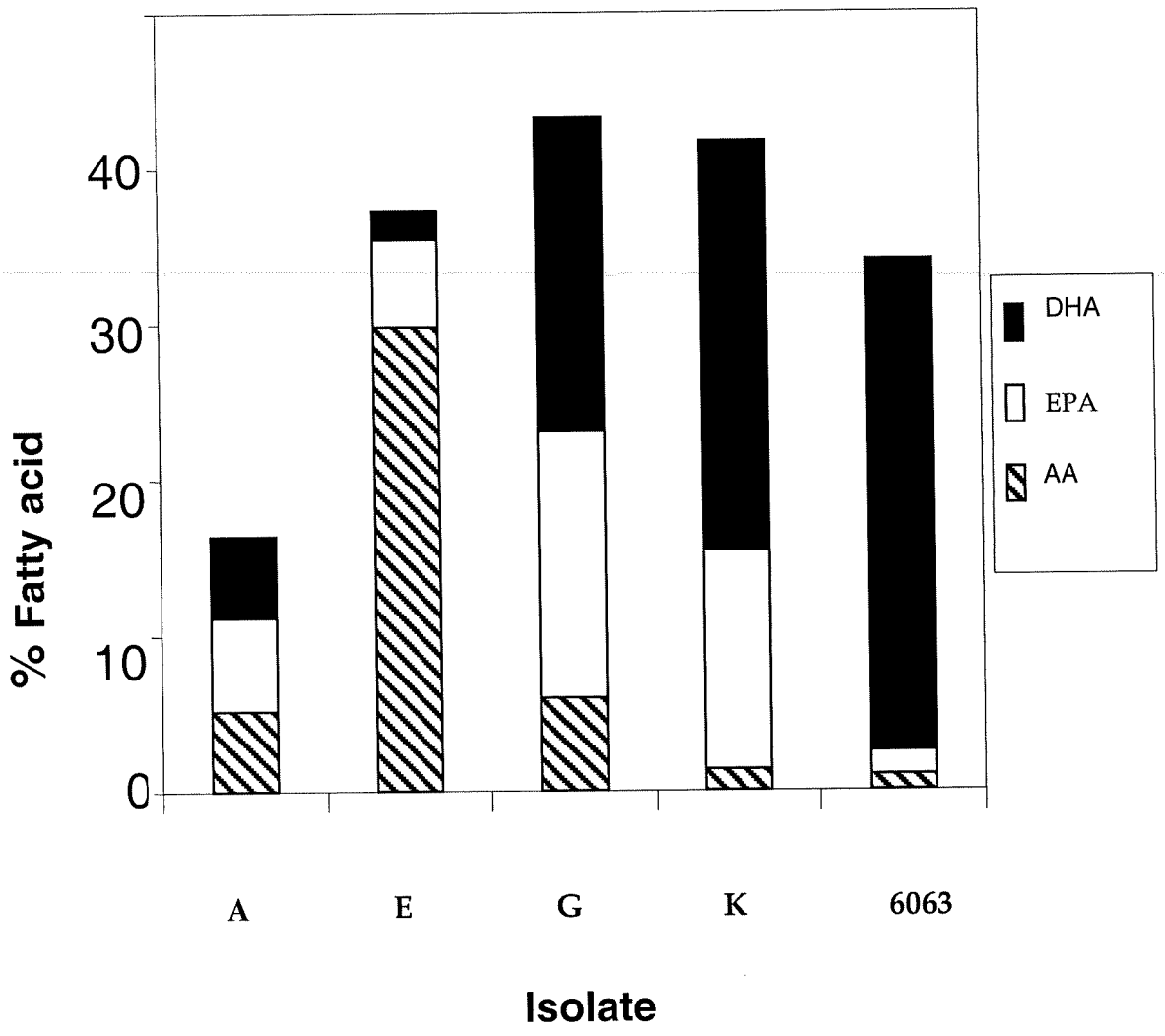

Figure 22.5. Composition of main PUFA in the oils of novel Australian thraustochytrids (Lewis 2001; Lewis *et al.* 1998a)

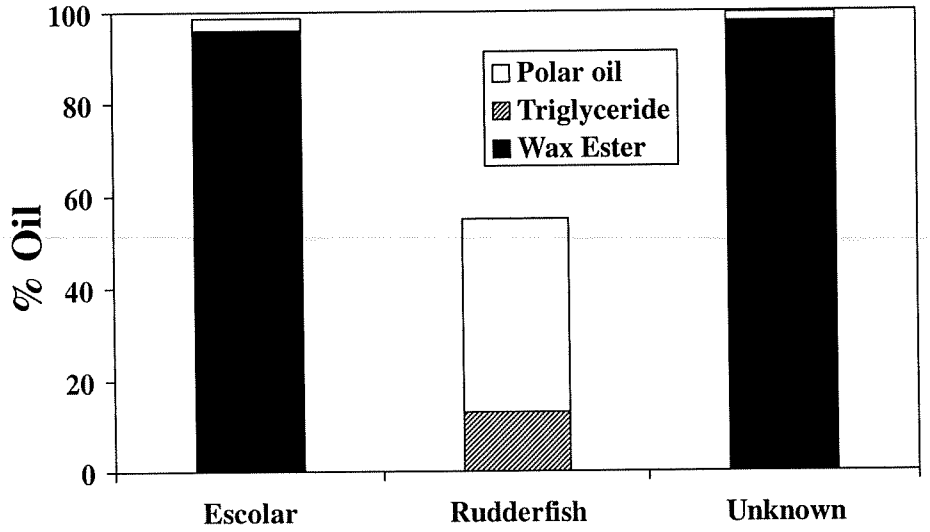

Figure 22.6. Lipid class composition of oil fishes (escolar and rudderfish) from Australian waters, including an unknown specimen associated with consumer illness.

Table 22.1. Summary of average content of LC omega-3 PUFA in wild-caught Australian seafood, with comparison to representative farmed species and other food groups.

Food group	LC omega-3 PUFA mg/100 g
Australian seafood (wild)	
Fish	**210**
Oysters	**150**
Prawns	**120**
Lobster	**105**
Farmed Australian fish	
Atlantic salmon	1930
Striped perch	2480
Silver perch	790
Barramundi	1970
Other food groups	
Turkey	35
Beef	22
Chicken	19
Lamb	18
Pork	0
Veal	0

Data for Australian seafood and non-seafood items from Nichols *et al.* (1998a)

Table 22.2. Lipid class and fatty acid composition (%) of fresh and cooked blue eye, and soup by-product

	Fresh	Pan fry	Deep fry	Steam	Grill	Microwave	Soup
%Total lipid	1.4±0.5	2.3±0.7	5.3±1.8	1.1±0.2	1.9±0.8	1.5±0.5	1.1±0.1
Lipid class (%)							
Wax ester	0.0±0.0	0.2±0.1	0.3±0.1	0.1±0.1	0.1±0.1	0.0±0.1	0.0±0.0
Triglyceride	14.1±8.6	58.1±11.5	84.4±2.9	5.7±4.8	26.8±14.4	8.5±9.0	94.1±0.5
Free fatty	0.3±0.1	0.2±0.1	0.3±0.1	0.3±0.1	0.2±0.0	0.2±0.1	0.5±0.1
Cholesterol	1.7±0.2	1.1±0.4	1.0±0.1	2.0±0.7	1.5±0.4	1.7±0.6	0.5±0.1
Phospholipid	83.9±8.4	40.4±11.0	14.1±2.8	92.0±4.2	71.4±14.7	89.5±8.5	5.0±0.3
Fatty acid (%)							
14:0	1.5±0.6	0.5±0.2	0.7±0.1	0.6±0.3	0.4±0.2	0.8±0.4	8.8±0.1
16:1ω7	0.8±0.1	0.5±0.1	0.6±0.1	0.8±0.4	0.5±0.2	0.9±0.7	2.3±0.0
16:0	18.3±1.8	10.2±1.5	21.5±0.2	18.5±1.4	11.4±0.7	18.2±1.1	27.0±0.1
18:2ω6 LA	0.9±0.1	12.8±1.1	43.3±3.2	2.3±0.9	10.7±1.1	0.9±0.1	3.4±0.0
18:1ω9 OA	13.6±2.3	41.1±4.5	18.8±0.1	12.1±3.1	35.7±2.6	11.3±5.0	27.3±0.1
18:1ω7	1.9±0.2	2.9±0.4	1.2±0.1	2.1±0.3	2.7±0.2	2.1±0.6	1.5±0.0
18:0	6.9±0.1	3.9±0.6	3.4±0.3	6.1±0.7	4.3±0.4	6.0±0.7	12.7±0.0
20:4ω6 AA	2.8±0.6	1.2±0.4	0.5±0.1	2.9±0.6	1.6±0.4	3.0±0.8	0.4±0.0
20:5ω3 EPA	4.3±0.8	2.2±0.3	0.8±0.6	5.0±1.6	2.8±0.5	5.2±1.5	0.7±0.0
20:1ω9	8.6±2.2	2.5±1.2	0.6±0.2	3.2±0.8	2.5±0.9	4.0±1.4	1.6±0.0
22:6ω3 DHA	27.2±3.7	13.9±3.0	5.0±2.1	36.0±5.3	19.4±3.3	36.6±6.2	2.4±0.0
22:5ω3	2.0±0.2	1.1±0.1	0.3±0.2	2.5±0.5	1.3±0.1	2.6±0.2	0.4±0.0
22:1ω11	2.7±1.0	0.7±1.0	0.1±0.1	0.6±0.3	0.4±0.4	0.9±0.8	0.6±0.0
other	8.6	6.4	3.1	7.1	6.1	7.6	10.7
Total SFA	27.9±1.5	15.8±1.9	26.5±0.3	26.5±1.7	17.5±1.1	26.3±1.4	53.4±0.1
Total MUFA	31.1±6.0	51.3±4.4	22.0±0.5	21.7±4.1	44.9±3.0	22.6±7.2	36.0±0.0
Total PUFA	41.0±5.0	32.8±2.6	51.6±0.8	51.8±4.1	37.5±2.5	51.2±5.7	10.6±0.0
Total ω3	34.9±4.3	17.7±2.8	6.3±2.7	44.4±4.8	24.0±2.9	45.2±4.7	3.7±0.0
Total ω6	5.6±0.7	15.0±0.5	44.1±3.0	7.1±1.2	13.4±0.7	5.8±1.3	4.1±0.0

Other includes 12:0,i14:0,14:1,i15:0,a15:0,15:0,16PUFA,i16:0,16:1ω9,16:2,16:1ω5,i17:0,17:1ω8/a17:0,17:0,18:3ω6, 18:4ω3,18:3ω3,18:2,18:1ω5,20:3ω6,20:4ω3,20:2ω6,20:1ω11,20:1ω7,20:0,21PUFA,22:5ω6,22:4ω6,22:3ω3,22:1ω9, 22:1ω7,22:0,23PUFA,24PUFA,24:1,24:0

Table 22.3. Lipid content and composition of cultured barramundi.

Lipid class	Percentage composition
Triglyceride	97.4±0.7
Free fatty acid	0.2±0.0
Cholesterol	0.2±0.0
Phospholipid	2.2±0.6
Oil content (wet weight basis)	10.0±1.2

23. BENEFITS

The study was designed to benefit Australian consumers, seafood marketing authorities, scientists examining the beneficial effects of consumption of seafood and fish oils from marine species, health and medical bodies including nutritionists, and ultimately the wide range of catching sectors of the industry by increased seafood sales.

The responses from all States and other organisations were very positive and supportive of the project, and assistance with obtaining samples was provided by many organisations.

Media and consumer group interest was evident throughout the project. There is now an increased level of awareness in Australia of the health benefits of omega-3 PUFA. As yet there is no recommended daily intake (RDI) for omega-3 PUFA in Australia. When nutritionists develop such RDIs, consumers can be more reliably informed about sources and benefits of omega-3 PUFA. At the present time, the consumption of Australian seafood remains a clear avenue for obtaining the beneficial LC-omega-3 PUFA.

In addition to this final FRDC report (1999/331), the results of this study are presented in the Guide *Seafood the Good Food II.* (Mooney *et al.* 2002). This Guide follows on from *Seafood the Good Food* (Nichols *et al.* 1998b). Results from the two Guides were also released in other forums including:

- marketing brochures of the NSW MFMA
- at national and international scientific conferences (see Appendix 3)
- a poster, flier and factsheet produced for facilitating the extension of results from Projects 1995/122 and 1999/331 (Appendix 3)
- the revised *Handbook of Australian Seafood* prepared by CSIRO, and the catering manual being prepared by Queensland Department of Primary Industries
- other reports being prepared by nutritionists conducting FRDC projects (e.g. Somerset and Bowerman, 1996/340; *Whats so healthy about Seafood?*).

Related FRDC funded projects (1994/115, 1999/369) worked closely with industry on the development of an Australian value added omega-3 oil (Nichols *et al.* 1997 a & b), and examination of other presently unutilized omega-3 containing oil resources (Nichols *et al.* 2001b). The establishment and appropriate archiving of a data base on the oil composition of Australian species will be of wider benefit to the Australian marine oils industry.

24. FURTHER DEVELOPMENT

The project has generated considerable interest from various client groups. We have received numerous requests for data on species, including various by-catch species or by-products. With the expansion of the CSIRO "Handbook" study to over 500 species, the opportunity to examine the oil composition of further species, particularly including emerging cultured species, is available and is recommended. This should include overseas species that are presently imported.

Further studies to determine both spatial and temporal variation in oil composition of commercial fish and other seafood are recommended, particularly for problematic species such as escolar (see below) and species where anecdotal evidence exists suggesting such variation affects product quality and value.

The continuing expansion of the Australian aquaculture industry indicates that farmed species will in the future capture a greater share of the domestic market. As aquaculture diets evolve, including with the possible incorporation of non-fish derived meal and oil, the need will exist to continue to determine the oil composition of seafood produced by the aquaculture industry. Care must be taken to ensure that the increasingly recognised health benefits of seafood, in particular the omega-3 PUFA provided via the marine food-chain, are not compromised. Research is recommended in this area.

An increasing number of incidences of illness associated with consumption of "rudderfish" has occurred recently. In this study, the high wax ester oil composition for fillet samples clarify the reported diarrhoeal effects on consumers. The purported "rudderfish" were in fact escolar. These results and the possible incorrect naming of the fillets suggest that consumers should be made aware of the oil type in these two groups and that strict use be made of recommended marketing names to avoid similar health issues. Oil composition results also supported genetic and taxonomic evidence (unpublished) that several undescribed species may exist in the rudderfish and escolar groups. Further specific taxonomic research on these groups is required, together with comparison of the oil profiles between and within (geographic and seasonal) species. In addition, insight into the physiological basis for the occurrence of these very different oils in the muscle of teleosts would be valuable.

Results obtained for several species suggest that it will be possible to use fatty acid profiles as biochemical tools to ascertain the environment from which any collected specimen has been recently feeding in. Such an approach could have application in the area of fisheries compliance. Further research is required before such tools can be used quantitatively.

Screening of seafood by-product derived oils for a range of bioactivities (e.g., anti-inflammatory, anti-cancer, antibacterial, other) would be a particularly useful avenue to pursue.

25. CONCLUSION

Oil profiles have been obtained for cultured and value-added seafood and for a further 79 species of Australian seafood species. The project team has worked closely with industry to examine the affect of farming and processing (e.g. cooking and other value-adding) on oil content and composition. Many of the chapters presented in this report were supplied to industry and other clents as summary reports (Appendix 3).

Particular emphasis has been placed on the highly beneficial LC-omega-3 PUFA oils. The main product of the research is a comphrensive Guide – *Seafood the Good Food II*, which has been prepared for use by the seafood industry, nutritionists and consumer groups in communicating the health benefits of both wild and farmed Australian seafood.

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The majority of samples analysed for this publication were provided by Gordon Yearsley through the Australian Seafood Handbook project. Other organizations, companies and individuals also kindly contributed samples to the projects: Mures Fish Centre provided fish and soup analysed in Chapter 16 . We thank Jill and George Mure and Paul Lynch for their help with the cooking performed in the Mures Upper Deck Restuarant, and for their ongoing interest in, and support of, the project. Tassal and Pivot provided Atlantic salmon samples (Chapters 10, 11, 12) and ongoing support, particularly Pheroze Jungawalla, Nick Burrows, Trevor Dix, Cindy Hope, Alex Brettingham-Moore and Al Bradley. Pheroze Jungawalla of Tassal in conjunction with Steve Battaglene of Tasmanian Aquaculture and Fisheries Institute (TAFI) provided farmed cultured trumpeter samples (Chapter 14). TAFI also provided abalone samples (Chapters 18, 19). Samples of the New Zealand green and Tasmanian blue mussels were provided by John Croft, MacFarlane Laboratories, and Vicki Wadley, Tasmanian Salmonid Growers Association (Chapter 21). Dave Borgelt of Bluewater Barramundi provided farmed barramundi samples (Chapter 13). Murray cod and silver perch were provided by Brendan Larkin of Australian Aquaculture Products (Chapter 16). Escolar and rudderfish samples were provided by Hunter Public Health Unit, NSW, and the South Australian Public Health Service (Chapters 8, 9). Kevin Williams and colleagues of CSIRO Marine Research (Cleveland) provided the cultured barramundi cod samples (data presented in *Seafood the Good food II*). Danny Holdsworth managed the CSIRO GC-MS facility.

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See also Appendix 3.

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28. APPENDICES

APPENDIX 1:

INTELLECTUAL PROPERTY

The project has generated interest from various client groups. During the project, we received numerous requests for data on shark species that to our knowledge had not been previously analysed.

The intellectual property generated during the project includes detailed oil compositional data on Australian seafood. Without specific details being provided here, some of these data already have been reported at national and international conferences, and provided to clients in report form (Appendix 3).

Interpretation of the oil compositional results has included examination of possible relationships between various biological and other parameters as indicated in preceeding chapters. Further intellectual property will be generated through similar use of the results from Project 1999/331 by CSIRO and other researchers as the data set becomes more widely available. At this stage, no direct commercial return is envisaged from other users of the project database. However, it may be appropriate to at least recover costs incurred for the provision of these data to other users.

APPENDIX 2:

STAFF

Mr. Ben Mooney	Res. Projects	BSc (Hons)	100%
Dr. Peter Nichols	Snr. Prin. Res. Sci.	BSc (Hons), PhD	20%
Dr. Nick Elliott	Snr. Res. Sci.	BSc (Hons), PhD	10%

APPENDIX 3:

Reports Prepared for Industry

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The project team also contributed to other scientific papers and reports that have been prepared as part of the FRDC Rock Lobster Enhancement and Aquaculture Subprogram (RLEAS) and other projects.

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Media and Related Material

October 1999. Sci-Files 99-10. "Health giving oils from fish waste" Interview produced by CSIRO and Pegasus Media and distributed to ABC radio.

August 2001. Science Now, Fresh science national competition, Melbourne. Ben Mooney was a national finalist, with a range of media interwiews, media clippings and a media presentation – "Chemistry sleuth fingers a slippery fish".

May 2002. Project 1999/331 press release; selected media clippings follow this section.

August 2002. Synergy, Hobart. "Fish Oils", featuring research from Projects 1999/331 and 1994/115, was one of ten science exhibits at the Science in Salamanca exhibition at CSIRO Marine Research Laboratories. The title of the fish oils presentation was "Imersion".

Media Release

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CSIRO Media Releases are also available from Newsline at: http://www.csiro.au

13 May 02 ref 02/89

SEAFOOD RETAINS HEALTHY OILS AFTER COOKING: CSIRO

There's more good news on the 'good oils' in seafood . . . cooking doesn't diminish the high level of beneficial oils found in seafood, according to research released today by CSIRO.

The research, funded by the Fisheries Research and Development Corporation, shows that Australian seafood – cooked, uncooked or processed, wild or farm-raised – is the best source of nutritionally-important omega-3 polyunsaturated fatty acids.

These fatty acids are needed to help prevent and treat heart disease and other disorders, but the human body only produces them in small amounts, so they must be obtained from the diet.

The research findings are presented in *Seafood the Good Food II*, a book released today (Monday) at Mures Restaurant in Hobart.

Information in *Seafood the Good Food II* is intended to help the seafood industry, nutritionists and consumer groups to communicate the health benefits of eating Australian seafood.

The book contains detailed oil profiles for 79 seafood species, as well as information on cooking, processing, aquaculture production and seasonal influences for selected species. It brings the total number of Australian seafood species profiled by CSIRO to nearly 300.

"Most Australian seafood is high in omega-3 polyunsaturated fatty acids and low in cholesterol." project leader Dr Peter Nichols of CSIRO Marine Research says.

"In fact, it contains 10–100 times higher levels of omega-3 polyunsaturated fatty acids than foods such as beef, chicken and lamb.

"But the nutritional value of farmed seafood, and the effects of cooking and processing on these beneficial oils were unknown, until now.

"We've determined that frying, grilling, steaming, microwaving and curing have no adverse effects. That must be good news for seafood lovers.

"And farmed fish such as Atlantic salmon, barramundi, silver perch and striped perch are high in omega-3 PUFA, and feeds can be tailored to increase this nutritional value," he says.

Nutritional studies continue to emphasise important links between seafood and human health.

"There is now good evidence in humans that the omega-3 polyunsaturated fatty acids in fish reduce heart attacks and particularly death from heart attacks," Dr Manny Noakes of CSIRO Health Sciences and Nutrition says.

"Animal studies have shown that this may be because omega-3 polyunsaturated fatty acids stop arrhythmias, the irregular heart rhythms that can lead to sudden death from cardiac arrest."

Dr Noakes says omega-3 polyunsaturated fatty acids from seafood act to lower triglycerides, one of the fats in the blood thought to contribute to heart disease.

They also appear to be involved in blood pressure regulation, platelet function and blood clotting, all of which may contribute to the prevention of heart disease.

The benefits of fish don't stop at heart disease prevention.

"Omega-3 polyunsaturated fatty acids from seafood may prevent stroke, may reduce the risk of premature births and may guard against prostate cancer," Dr Noakes says.

"They are also helpful in the treatment of rheumatoid arthritis and some recent studies indicate a benefit in some forms of depression."

Steve Gill of the Master Fish Merchants Association of Australia says the findings are great news for the seafood industry.

"Our association will be spread the message through distributing posters and brochures explaining the results of the study to fish retailers throughout the country," he says.

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***Seafood the Good Food II* is available from CSIRO Publishing 1800 645 051.**

Shepparton News Wednesday 15/5/2002
General News Page 18

In any form, fish oil reduces heart disease

The good oil on fish oil is that it's really good for you.

The better news, according to a CSIRO study released this week, is that normal cooking and processing don't affect the goodness and the oil from farmed fish is just as good as that from wild fish.

What's more, Australian fish generally have more of the good oil than their Northern Hemisphere counterparts.

The study, published in book form as *Seafood the Good Food II*, found that Australian seafood in whatever form, even canned tuna or supermarket fish fingers, was the best source of nutritionally important omega-3 polyunsaturated fatty acids.

CSIRO nutritionist Manny Noakes said while research was continuing on the beneficial effects of omega-3, it significantly helped to reduce heart disease.

Dr Noakes said it may also prevent strokes, reduce premature births, help child development, guard against prostate cancer and assist in the treatment of rheumatoid arthritis.

Two fish meals a week are recommended, but there's no limit.

The good oil on seafood is get cookin'

THE good oil on seafood is that it doesn't lose its good oils through cooking.

This new research is contained in the book *Seafood the Good Food II* launched yesterday at Mures in Hobart.

Research by the Fisheries Research and Development Corporation has shown that fish, cooked or uncooked, is the best source of omega-3 polyunsaturated fatty acids.

These fatty acids are beneficial in reducing and treating heart disease and other disorders.

Because the human body produces them in small amounts, they must be obtained through diet.

Seafood the Good Food II is intended to help the seafood industry, nutritionists and consumer groups sell Australian seafood.

"Most Australian seafood is high in omega-3 polyunsaturated fatty acids and low in cholesterol," project leader Peter Nichols, of the CSIRO, said.

"In fact, it contains 10-100 times higher levels ... than foods such as beef, chicken and lamb.

"But the nutritional value of farmed seafood, and the effects of cooking and processing on these beneficial oils were unknown, until now.

"We've determined that frying, grilling, steaming, microwaving and curing have no adverse effects. That must be good news for seafood lovers," he said.

HEALTHY: CSIRO scientist Nick Elliott shows a selection of fish that are high in Omega-3. Picture: RAOUL KOCHANOWSKI

Sydney Morning Herald Tuesday 14/5/2002
General News Page 5

The good oil on healthy eating is slender tuna

Andrew Darby
in Hobart

You might not have heard of it, but if you are into healthy eating you should think about tucking into some Australian slender tuna.

The little-known fish, found in warmer waters from Queensland to northern NSW, has been rated the nation's healthiest seafood dish, thanks to its high level of nutritionally important fish oils.

The fish made the top of a list compiled by the CSIRO showing the oil content and composition of 79 seafood species.

The small migratory tuna can pack 3700 milligrams of omega-3 polyunsaturated fatty acids per 100 grams. That compares with an average of 235 mg for fin fish, 18mg to 19mg for chicken and lamb, and zero for pork.

Omega-3 polyunsaturated fatty acids from seafood oils are regarded as important because of their wide range of potential health benefits. They can guard against coronary heart disease, stroke and rheumatoid arthritis. They may also help against some forms of cancer, and promote infant brain development.

The CSIRO also released advice that the cooking and processing of fish did not adversely affect their levels of omega-3s. Researchers believe antioxidants in the oils prevent them from breaking down when they are cooked, and the relatively brief time needed to cook fish may also have an influence.

The CSIRO's project leader, Peter Nichols, said the "good oils" of fish such as slender tuna could be consumed freely. "I'm not aware of any study that shows you can eat too much."

The slender tuna is generally taken as a by-catch by east coast and northern trawl fishermen.

Its extraordinarily high oil content is a result of its prey, which includes krill, squid and smaller fish such as the jack mackerel. The level of omega-3s can vary depending on the tuna's recent behaviour.

"If it's caught just after a big feed, then the good oil levels are very high," said a CSIRO biologist, Nick Elliott. "But if it's caught at the end of a migration, then its oil levels could be lower."

Next highest in omega-3s are the more readily available farmed fish, such as the striped perch (2500 mg), atlantic salmon and barramundi (each about 2000 mg).

A snappier line in fish

What you see is what you'll get

By KAREN COLLIER, consumer reporter

SUCCULENT sea perch may soon be no more ... by that name, at least.

Soon they will be called tropical snapper, falling into line with years of shopfront slang.

Proposed changes to the Australian Fish Names List coincide with moves for a further blitz on seafood labelling frauds or errors.

Pacific dory, a common sales name for a Vietnamese catfish, would be renamed basa.

Only one fish species would be called blue grenadier, with the rest of its close relatives usually hooked overseas dubbed hoki.

Duckbill, the internationally recognised name for a fish imported from Argentina, would be dumped from our shores.

Importers worried about the name's connection with platypus prefer the product to be known as South American flathead.

Official marketing names for hundreds of local and imported fish are expected to be enshrined in national food laws late next year, making it harder for retailers to deliberately or accidentally mislead consumers.

"We want people to be confident what they are buying is what they are getting," Seafood Services Australia senior technical officer Alan Snow said.

Seafood Services Australia wants to alter or add 13 fish names to the industry's seafood title bible and has called for public submissions.

The move would officially recognise names for several imported fish that are fairly new to the market, as well as create separate names for others to avoid consumer confusion.

Mr Snow said sea perch had been commonly sold as snapper despite a ruling two decades ago and it was time for that to be recognised.

He said a particular species of sea perch, lutjanus bohar, that was sometimes sold as bohar, would get the separate title red bass.

This was because the fish was a potential source for a toxin that caused ciguatera poisoning. The main symptoms include hot and cold flushes and nausea.

Other mooted changes include creating the name oilfish for a type of escolar that has a laxative effect on some people. The species, ruvettus pretiosus, is often confused with rudderfish or butterfish.

Distinct names for premium products normally lumped in with generic labels have also been proposed. These include white pomfret, giant squid and royal basa.

Net link: *www.seafood-services.com.au*

THE good oil on fish oil is ... it's really good for you.

The better news, according to a CSIRO study released yesterday, is that normal cooking and processing don't affect the goodness — oil from farmed fish is just as good as that from wild fish.

What's more, Australian fish generally have more of the good oil than their northern hemisphere counterparts. The study, published in book form as *Seafood the Good Food II*, found that Australian seafood in whatever form — even canned tuna or supermarket fish fingers — is the best source of important omega-3 polyunsaturated fatty acids.

CSIRO nutritionist Manny Noakes said that while research was continuing, omega-3 significantly helped to reduce heart disease. It may also prevent strokes, reduce premature births, help child development, guard against prostate cancer and assist in the treatment of rheumatoid arthritis. It may even benefit some forms of depression.

A cultured diet

By 2020, most of the seafood we eat will be farmed, or 'cultured', rather than caught from the sea. Studies of farmed fish show that they generally contain high oil and PUFA levels, and feeds can be tailored to increase this nutritional value.

Serving it up

Frying, grilling, steaming and microwaving have no adverse effects on omega-3 PUFA levels in seafood. Nor do hot and cold smoking, or salt and brine curing. Even the by-products from filleting, used in minces and stocks, are PUFA rich. Oils used in deep frying should be clean and regularly replaced.

Some finfish and shellfish vary in their oil content with location and season, but the differences have little effect on their nutritional quality.

More good oil

Studies by CSIRO of the good oils in Australian seafood have been funded by the Fisheries Research and Development Corporation. Results are summarised in *Seafood the Good Food* and *Seafood the Good Food II.* The books are available from CSIRO Publishing, freecall 1800 645 051.

Seafood enquiries

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THE MASTER FISH MERCHANTS' ASSOCIATION OF AUSTRALIA

FISHERIES RESEARCH & DEVELOPMENT CORPORATION

CSIRO MARINE RESEARCH

seafood

brimming with good oil

Fresh, cooked and processed, caught wild or grown on a farm . . . seafood gives you the goodness of omega-3

Average omega-3 PUFA content (mg/100 g wet weight)

fish	235
oysters	150
prawns	130
lobster	105
turkey	35
beef	22
chicken	19
lamb	18
pork	0
veal	0

Lasting goodness

Marine scientists at CSIRO have discovered that Australian seafood contains 10–100 times higher levels of omega-3 polyunsaturated fatty acids, or PUFA, than foods such as beef, chicken and lamb.
They also know it makes no difference what path our seafood takes to the table. Caught from the ocean, or raised on a farm; served however our heart desires; the omega-3 goodness is retained.

Food for the heart

Omega-3 PUFA help to prevent high blood pressure, coronary heart disease, heart-rhythm disorders, blood-vessel deterioration, and sudden death from heart attack.

They also appear to promote infant brain and retina function and development, to prevent stroke and rheumatoid arthritis, and may guard against some forms of cancer.

The two most important omega-3 PUFA are docosahexaenoic acid and eicosapentaenoic acid, known simply as EPA and DHA.
Our bodies make only small amounts of these fatty acids, so we need to source them from our diet, and the best source is seafood!

Seafoods also contain the omega-6 fatty acid arachidonic acid, or AA, which promotes growth and general wellbeing.

How does PUFA in seafood vary?

There are as many variations of PUFA levels in seafood as there are, well, fish in the ocean, and the task of measuring them is well under way.

Studies of 300 popular finfish, shellfish and crustaceans have found most Australian finfish are high in PUFA and low in cholesterol. They also contain higher relative levels of DHA than fish from Northern Hemisphere waters.

seafood

brimming with good oil

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Nutritional Value

OF AUSTRALIAN SEAFOOD II

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