FATS FOR THE FUTURE

The Proceedings of the
International Conference on Oils, Fats and Waxes, Auckland, 1983

Editors
S. G. BROOKER
A. RENWICK
S. F. HANNAN
L. EYRES

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INTRODUCTION

The contents of these Proceedings are, in essence, the papers which were presented at the International Conference on Oils, Fats and Waxes held at the University of Auckland on 13-17 February 1983. Some authors have taken this opportunity to expand their papers in order to give a wider review of the subject; in some other cases an abstract was felt to be all that was necessary.

The Conference was the first of its kind in New Zealand, and indeed, as far as we are aware, the first in the Southern Hemisphere (but only just, as one was held in 1981 in Kuala Lumpur, 3° North). We believe that the quality of the papers given and the generally favourable comments received from delegates should give the lie to the current fashion of regarding the 'South' as an impoverished intellectual desert.

With the grand total of 170 persons involved, the conference was a small one which made for a close relation between the delegates at both the technical and social events. The ratio of one overseas visitor for every two locals was a good one, especially as the visiting contingent included a number of plenary speakers of world reputation.

The Chairman first generated interest in a proposed conference from representatives of local companies processing oils and fats. In the subsequent workings of the Organizing Committee, the commercial experience of Dr Laurence Eyres (Technical Programme), Mr Graham Ryburn (Registrar) and Mr Ken Burnett and Mr Gordon Winward (Treasurers) combined with their efficiency and enthusiasm contributed a great deal to the smooth operation which culminated in February 1983. They were well supported by representatives of the Department of Scientific and Industrial Research, the Ministry of Agriculture and Fisheries, the Dairy Board and other commercial interests, who all made a useful contribution as well as involving the concerns the represented. Their names are listed elsewhere in this work.

Also welcome was the early interest manifested by Profs. Renwick (Biochemistry) and Scott (Medicine) which had several important effects. It was their philosophic use of the crystal ball that led to Prof Sune Bergstrom of Stockholm being enlisted as a plenary speaker before the announcement of his Nobel award in September 1982, and these two Professors were also able to secure funds to bring Profs Bergstrom, Hegsted and Birkbeck to Auckland. But the greatest effect of their interest was the widening of the scope of the Conference so that it became a symposium on lipids in the widest connotation of the term.

Lipids are a division of organic chemistry, but there is much less research on their chemistry in the Universities and other places of research than their importance in our national health and economy warrants. It was good therefore to have Professor R C Cambie, head of the Department of Organic Chemistry at Auckland as Vice-Chairman, where he played an important part. He was also instrumental in securing a grant from the Royal Society of N.Z. which coagulated various ideas into a firm commitment to hold the Conference.

Another worker was Dr Sharon Hannan, who not only worked hard on the Committee, but also on the work of editing, including acting as liaison officer between the editors and the publisher.

Opportunity was taken during the official dinner to present engraved trays to Dr F B Shorland, O.B.E., the doyen of lipid scientists in New Zealand, and to the Chairman, both of whom, as befitting their seniority, presented papers on historical aspects of fats, which are included in this book.

It is a pleasure to record our thanks to many organisations who contributed in cash or kind to the financial success of the Conference. Special mention should be made of Dr Cecil Johnson, of the D.S.I.R., Palmerston North, who secured $US1000 from each of three United States companies which enabled Prof Ralph Holman (and Mrs Holman) not only to attend the Conference, but to lecture in other parts of the country as well.

S. G. Brooker.

Opening Address : International Conference on Fats, University of Auckland, February 14th 1983

Dr the Hon Ian Shearer

Minister of Science and Technology, Government of New Zealand.

Ladies and Gentlemen, Fellow Scientists,

It is my great pleasure today to open this International Conference on Oils, Fats and Waxes held for the first time ever in New Zealand.

I would like first to congratulate our plenary speaker, Professor S Bergström, Rector of Sweden's Karolinska Institute, on the award of the 1982 Nobel Prize for Medicine. I am informed the award was for his work on that interesting group of chemicals known as prostaglandins. That point alone indicates to me the tremendous progress that has been made in many fields of research over the last decade. When I was studying for my PhD in Reproductive Physiology at Nottingham University in the late 1960s and early 1970s, the study of prostaglandins was very much a new and rapidly emerging field. Today we have with us a Nobel Prizewinner in that very field.

Professor Bergstrom, in his welcoming address to the first Karolinska Symposium, in 1969 to be exact, had this to say: "It is, however, of special importance in the present case that all new information becomes available not only to scientists now active in the field, but also to other biochemically oriented scientists and organisations in order to stimulate new scientists to enter the field and to get pertinent results rapidly into the teaching of the medical schools."

While this conference is dealing with a different topic, the message is very much the same, and I extend a warm welcome to Professor Bergstrom, to the other plenary speakers, and to the distinguished scientists who have gathered here from many parts of the world in order to provide New Zealand scientists with that same stimulation.

We meet at a time when world commodity prices are depressed, and commercial returns for oil and fat products are generally not good. But the variety of topics to be discussed during this conference is an encouraging indication of international efforts to increase consumption. This is being done by research into more efficient production of oil seeds, and oils and fats in general; by the better use of traditional products in the development of new materials; and by nutritional studies to improve human and animal health.

Fats in their broadest meaning have been a major component of New Zealand's export trade, and one of the foundations of our prosperity. They have been so since we first started sending our butter, cheese, and sheep meats to the markets of Europe, 100 years ago. But international competition and changing dietary habits have changed the pattern of trade for our primary products over the past two decades.

High intakes of fat, and particularly animal fats, have been linked to obesity and heart disease. The result has been a steady decline in international levels of fat consumption, and moves to replace animal fats with unsaturated fats. With this, we have had to face the loss of traditional markets when Britain joined the EEC, and the problems of over-production from competitor countries with more heavily protected or subsidised farm production. But we put our traditional Kiwi adaptability and innovative skills to work, and began the search for more effective or profitable uses for our fat production, and for greater competitiveness in producing animal fat products. The results of that drive to diversify are shown to maximum advantage by our enterprising dairy industry. Until the mid-1960s, the exports of New Zealand's dairy industry were limited almost entirely to salted, non-lactic butter and cheddar cheese, 90 percent of which was exported to the United Kingdom. Today the same industry produces some 2,000 different products ranging from anhydrous milkfat, whole milk powders and caseinates, to fancy cheeses, tinned butter, and infant foods. Dairy products now go to well over 100 countries, and in 1981 earned New Zealand over $\$1.3 \times 10^9$ in exports.

New Zealand's sheep meat industry has been facing similar pressures from loss of traditional markets, and competition from poultry and veal which have a lower fat-to-protein ratio. Probably 30 percent of our export lamb now has more fat than desirable for modern markets. The industry is adapting to changing conditions with research to produce lean animals, and research to produce higher protein-yielding sheep carcasses by breeding, nutritional management, or possibly genetic engineering. Meat research scientists have developed a low-temperature rendering process that has the potential to produce higher grades of tallow, and more importantly, to convert low-grade or fatty sheep carcasses to separated edible tallow and edible protein concentrates.

Our DSIR is currently working on a process to convert inedible tallow to methyl esters which are potential sources of a wide range of chemicals and possibly food products. An investigation is also in hand by the Liquid Fuels Trust Board on the suitability of methyl esters as diesel fuel extenders. New Zealand scientists are also working to develop other sources of fats with commercial potential from fish, wool, forestry, peat, lignite, and plants.

For example, there are good prospects for the liquid wax from the Orange Roughy fish, a fish unknown in New Zealand waters three years ago, but now caught in large numbers off the Chatham Rise. The Orange Roughy harvest is currently 5 to 10,000 tonnes a year with a potential yield of 3 to 4,000 tonnes of oil. This oil can be used as a substitute for whale oil or jojoba bean oil in cosmetics, lubricants and the like, although this is a competitive market. The DSIR estimates its potential return to New Zealand at about $\$3 \times 10^7$ per annum.

Our forest industry produces as a by-product, Tall oil, long known as a by-product of the wood pulping process. Now a consortium of the two New Zealand wood pulp producers have a joint project under way to fractionate and market Tall oil products. Its production will be up to 10,000 tonnes per annum.

The development of new processes to recover lanolin from wool scouring effluent have increased recovery to over 95 percent. Material surplus to what can be sold is currently being used as fuel oil in the scourer process, but my hope is that our researchers will soon develop high-value products to increase our earnings from this waste material.

A very early industry in the history of New Zealand was the export of Kauri Gum for varnish. Changing technology put an end to this industry, but investigations are now under way to develop a new industry based on the same source-Kauri peatlands. High value peat waxes can be extracted from these deposits, together with rosin acids derived from Kauri. A pilot project is underway between two New Zealand companies to finalise development work for what is thought may well be a large export market. The use of vegetable oils as fuel substitutes other than in cases of national emergency will depend on the cost of production in comparison with other fuels.

Conferences like this one are a valuable source of knowledge as to the viability and potential for such materials. I have attempted in the course of this address to give you some insights into the relation of oils and fats to New Zealand's economy. I have spoken of our traditional dependence on fats for prosperity and the problems we have faced because of agricultural surpluses and the decline in world demand for animal fats. I have described our successful search for other uses for our fats, new sources of fats, and new markets. My hope is that this address has provided some useful perspectives both for your own research and for this conference.

I now have much pleasure in declaring this International Conference on Oils, Fats and Waxes, open.

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INDUSTRIAL SECTION

Marine Lipids

Professor R. G. Ackman

Canadian Institute of Fisheries Technology, Technical University of Nova Scotia
P.O.Box 1000, Halifax N.S., B3J 2X4.

An 'oil' can mean different things to different people. Merck's Index, 7th edition[1], lists about nine pages of oils (from oil of amber, rectified, to oil of yarrow). Only one, oil of mustard, is of the type familiar to contemporary lipid scientists. The reason is clear, since it is described as a 'fixed' oil. The others are of course all 'essential' or 'volatile' oils, with components as diverse as benzaldehyde and geraniol.

A 'fixed' oil is reasonably well understood to be a triglyceride. Although some would say that the lauric acid of the coconut palm is a borderline case for volatility, the other fatty acids with which this paper is concerned are decidedly involatile even if not bound in threes as triacylglycerols. Although the variety of fatty acids and derivatives in fats of invertebrate marine life is frequently surprising[2,3] this extends to marine mammals as well. There are families of toothed whales who have adapted the already biochemically available isovaleric and isobutyric acids as major components of the fats in their bioacoustic organs. These unusual fats include both the triglycerides and wax esters[4].

One of the historic exceptions to the triglycerides making up something like 98-99% of marine oils is, of course, sperm whale *(Physeter catodon)* oil, which has long been known to contain the valuable component spermaceti[4,5]. This is defined by Merck as 'chiefly cetyl palmitate.' We shall return to wax esters, a topic currently of keen interest to New Zealand lipid scientists, but having considered oils briefly, we must also define the term 'lipid'.

Originally, a lipid was anything soluble in certain organic solvents, for example, chloroform. Traditionally benzene, ethyl alcohol and diethyl ether were also acceptable, especially if hot. Unfortunately most such solvents were incompatible with water, a normal ingredient in the tissue of marine organisms. The custom of oven drying or freeze drying samples before lipid extraction persists to this day, usually with disastrous results such as a recent (1982) report of only 0.11% of 'oil' in the muscle of the Alaska pollock *Theregra chalcogramma*[6]. This is likely only one-fifth of the minimal probable content. The problem of breaking up the delicate combination of lipid, water, and protein without destroying the lipid was in fact only solved with the homophasic system of chloroform, methane and water usually referred to as the 'Bligh and Dyer' method[7]. Although so widely used that it has been honoured as a 'Citation Classic'[8], we should reflect that this method was developed from the 'Folch' method in a fisheries laboratory for the purpose of reliably extracting the low levels of lipid present in the flesh of the Atlantic cod *Gadus morhua*. Despite twenty-five years of work on this topic[9], the method is still being refined for study of almost exactly the same problem, and it is a salutary lesson to all of us that free fatty acids can be lost from the system if due care is not taken[10].

This presentation is too short to go into the functions of liver, gill, nervous systems and other interesting areas of physiological biochemistry. The most basic lipid however must consist of the widely accepted phospholipid bilayer[11,12].

Associated with these functional lipids[13] are the triglycerides, sterols and sterol esters, and small (5-15%) proportions of these lipids may be thought of as also functionally involved with membranes. In Table I we can compare the total lipid and some of the lipid classes, and three specific phospholipids, in a mollusc, two crustacea, and three fish.

Table I. Comparison of total lipid and some lipid fractions in flesh of three shellfish and of three fish.

Lipid and fraction	Abalone (14) *Haliotis midae*	Pink Shrimp (15) *Pandalus montagui*	Queen Crab (16) *Chionoecetes opilio*	Hake (17) *Merlucius capensis*	Cod (18) *Gadus morhua*	Trout (19) *Salmo gairdneri*
Total lipid, g/100g	1.1	1.39	0.75	1.55	0.59	3.5
Polar lipid, g/100g	0.69	0.62	0.56	0.46	0.52	0.97
% Phosphatidyl choline	37	44	62	63	69	58
% Phosphatidyl ethanolamine	32	34	28	21	19	21
% Sphingomyelin	2	4	5	4	-	
Neutral lipid, g/100g	0.3	0.77	0.2	1.0	0.1	2.5

Considering that the abalone analysis dates to the early days of chromatography on silica acid it is a credit to A.J. deKoning that he found phosphatidyl choline exceeded phosphatidyl ethanolamine in both the mollusc and the fish[14,17]. This proportion of the two major polar lipids is a characteristic of the polar lipids of marine organisms, including the freshwater fish[19]. The basic cellular composition of muscle tissue of normal and healthy molluscs, crustacea and fish determines the minimal lipid present, which from numerous studies[2,3,20-23] may be set at a minimum of about 0.6% of wet weight except in the case of 'red' or 'dark' muscle of fish. In these metabolically active tissues[24,25], for example the lateral line muscle of cod, total polar lipid is normally twice as high[21,22] as in the light muscle used for more languid slow-speed swimming. Starved molluscs or cod have obviously slightly lower levels[23], but in the case[26] of the Atlantic black halibut *Reinhardtius hippoglossoides* the lipid in gelatinous meat was only reduced from 11.62% to 2.45%. Polyenoic acids are stated to have increased from 10 to 45% of the total fatty acids, but in this analysis 22:1 and 20:5 are confused. The gelatinous meat is deproteinized, but the residual 2% lipid indicates that there may still have been some depot fat left.

Table II. Important and interesting fatty acids, in particular lipids of some fish species. (TG : isolated triglyceride, PL : polar lipid, mostly phospholipids).

	Atlantic redfish(*) *Sebastes marinus*		Atlantic sturgeon (57) *Acipenser oxyrhynchus*				Indian oil sardine (53) *Sardinella longiceps*	
Sample	Nape	Notch meat	Orange Layer	Muscle	Liver	Liver	Muscle	Skin
Lipid	TG	TG	TG	TG	TG	TG	(6.0% lipid)	(27.4% lipid)
Fatty acid								
14:0	5.6	5.1	3.8	3.5	2.4	1.5	9.4	10.2
16:0	11.9	13.1	15.2	14.8	18.3	25.7	18.6	18.2
18.0	1.9	2.3	6.3	5.7	4.7	11.8	6.1	6.1
Total	19.4	20.5	25.3	24.0	25.4	39.0	34.1	34.5
16:1	13.8	13.1	2.0	3.1	2.8	1.7	9.7	13.6
18:1	16.5	18.5	30.0	32.4	36.2	13.7	9.3	10.0
20:1	13.7	11.7	7.9	5.3	4.3	3.8	1.1	1.0
22:1	19.9	15.2	1.0	0.4	0.4	0.8	1.2	0.6
Total	63.9	58.5	40.9	41.2	43.7	20.0	21.3	26.2
18:2ω6	0.6	0.6	0.8	0.8	0.7	0.5	2.5	3.8
18:3ω3	0.2	0.3	0.4	0.4	0.2	0.2	1.4	1.5
18:4ω3	0.9	0.8	1.2	1.1	0.5	0.1	2.3	2.1
20:4ω6	0.1	0.1	1.3	1.4	0.8	6.9	2.7	2.1
20:5ω3	7.4	9.6	10.2	10.2	7.0	7.4	15.2	14.5
22:5ω3	0.2	0.5	2.5	2.6	4.9	2.9	2.8	2.7
22:6ω3	2.4	4.5	3.3	4.4	5.1	16.5	11.5	7.1
Total	11.8	16.4	19.7	20.9	19.2	34.5	38.4	33.8
Total, these components	95.1	95.4	85.9	86.1	88.3	93.5	93.8	94.5

*R G Ackman, unpublished results.

Before proceeding to discuss depot fat in detail, it is well to note that free fatty acids do not normally amount to more than about 1% of this minimal cellular lipid or indeed of total lipid. All reports of high proportions of free fatty acids should be critically reviewed in terms of post-mortem lipid hydrolysis, often due to delayed freezing or poor frozen storage[9,27-29]. In some cases of full stomachs, post mortem autolysis from digestive enzymes is a possible contributing factor[30].

Depot fats are normally reviewed in terms of triglycerides[31,32], but in the case of molluscs, glycogen is the chief energy resource. In the last two decades it has become widely accepted that in many copepods, and also some euphausiids, wax esters as well as triglycerides are important in depot lipids[33-40]. Wax esters in lipids of mesopelagic fish are biosynthesized *de novo*[35], and their role is more obscure. It is possible that the physical properties[40-43] could be an alternative to a purely nutritional role. Only thirteen years ago, in 1970, we proposed that 22:1 had a definitive role in the buoyancy of clupeids such as herring[44], a view reconsidered when it became clear that the docosenoic acid in question was mostly 22:1*w*11 isomer and of exogenous origins[45-48]. One argument against reading too much of a specific role into depot fats of fish is the different distribution of depot fats. In the gadoids such as the cod *Gadus morhua* the liver is the only fat storage organ, while in the clupeids as much as half of the depot fat is in the subcutaneous flesh[21,32,49-53]. In certain fish most, or a part of the lipid may be in the skeleton[54-56]. In most fish with depot fats in different locations the triglyceride tends to have a uniform fatty acid composition independent of distribution in the animal (Table II). However, where this is locally associated with a biochemically active functional lipid (e.g. liver phospholipid of sturgeon), some influence of the polar lipid will often be found, (e.g. the 16:0, 20:5*w*3 and 22:6*w*3 in the liver triglyceride of the sturgeon).

Table II shows the limited number of fatty acids making up about 90% of the total and adequate to describe marine lipids in most cases. As few as eight fatty acids are sufficient[58]. Biochemically interesting fatty acids such as the 20:2 and 22:2 NMID (non-methylene-interrupted dienoic acids), or *trans*-6-hexadecenoic acid, are basically formed in marine invertebrates[2,3] and accumulate in depot fats of higher organisms such as fish[57], or some marine turtles[59], but usually are not more than 1 or 2% of total fatty acids. Our work with odd-chain fatty acids in smelt *Osmerus mordax* has shown that a seasonal heavy deposition of a range of unusual fatty acids from a dietary source in the form of small crustacea is soon reversed, and the species reverts to normal[60]. This particular phenomenon has also been observed in the mullet *Mugil cephalus*, ostensibly a vegetarian[61], so the source of biochemically inert fatty acids is not important. Octadecapentaenoic acid is a similar case of an unusual fatty acid of plant origin sometimes accumulated by invertebrates[62,63], but not readily observed in depot fats of higher species of fish. We can conclude that the fatty acid biochemistry of marine organisms is highly tuned to certain basic patterns on which may be superimposed species needs arising from local foods, ecological factors, or reproductive needs.

It is difficult to get excited about the role of the even number, straight chain, saturated fatty acids in marine organisms. All are freely available from food and can be biosynthesized *de novo*. The monoethylenic fatty acids are more interesting, although 16:1*w*7 (palmitoleic) acid and 18:1*w*9 (oleic) acid are also common and have similar origins. However, when the other monoethylenic isomers such as 18:1*w*7 (*cis*-vaccenic acid), and especially the 22:1 isomers, are considered[45,46] much useful information can be extracted from the monoethylenic fatty acids[47,48].

This paper does not permit an in-depth listing of fatty acids of marine oils and lipids, especially as recent publications include such tabulations[22,48,64]. The reproduction of a table published[65] by Itabashi and Takagi (Table III) will serve several purposes, for example to illustrate the relative simplicity of the fatty acids of seaweeds (and in general many marine algae). These organisms have already been reviewed elsewhere[64]. Table III lists only 23 fatty acids for the seaweed *Undaria pinnatifida* as against 86 component peaks in the analyses of marine animal lipids.

Table III. From Itabashi and Takagi.

Peak no.	Fatty acid	Retention data RRT	ECL	Composition (wt%) Sardine *Sardinops melanosticta* total lipids	Pollack *Theragra chalcogramma* total lipids	Zooplankton copepod[b] wax esters	Seaweed *Undaria pinnatifida* total lipids	Sea urchin *Strongylocentrotus intermedius* total lipids
1	12 : 0	0.1026	12.00	0.12	0.02	0.19	ND[a]	0.03
2	*iso*-13 : 0	0.1276	12.57	0.01	ND	0.01	ND	0.02
3	13 : 0	0.1508	13.00	0.07	0.01	0.09	ND	0.03
4	*iso*-14 : 0	0.1864	13.55	0.04	0.01	0.11	ND	0.02
5	14 : 0	0.2221	14.00	7.46	4.08	21.33	2.45	11.35
6	14 : 1 (n-9)	0.2400	14.21	0.13	0.09	0.40	ND	0.02
7	14 : 1 (n-7)	0.2444	14.26	0.04	0.01	0.03	ND	0.04
8	14 : 1 (n-5)	0.2587	14.41	0.04	0.11	0.13	ND	1.16
9	*iso*-15 : 0	0.2721	14.55	0.15	0.14	0.25	ND	0.15
10	*anteiso*-15 : 0	0.2872	14.69	0.27	0.04	0.15	ND	0.11
11		0.3033	14.84	0.01	0.01	0.02	ND	ND
12	15 : 0	0.3220	15.00	0.63	0.22	0.45	0.19	0.31
13		0.3425	15.15	ND	0.01	0.09	ND	0.01
14		0.3653	15.31	0.01	ND	0.05	ND	0.03
15	*iso*-16 : 0	0.3952	15.51	0.15	0.07	0.07	ND	0.08
16		0.4397	15.78	0.01	ND	0.03	ND	0.05
17	16 : 0	0.4808	16.00	21.97	11.23	21.82	10.86	19.71
18	16 : 1 (n-11)	0.4996	16.11	0.06	0.03	0.06	ND	0.09
19	16 : 1 (n-9)	0.5138	16.19	0.35	0.19	0.24	0.08	0.10
20	16 : 1 (n-7)	0.5343	16.30	7.93	7.28	5.75	0.18	8.61
21	16 : 1 (n-5)	0.5513	16.39	0.11	0.08	0.27	1.81	2.46
22	*iso*-17 : 0	0.5789	16.52	0.20	0.12	0.12	ND	0.05
23		0.5991	16.62	0.12	ND	0.20	0.07	0.11
24	*anteiso*-17 : 0	0.6093	16.67	0.18	0.18	0.07	ND	0.04
25		0.6530	16.86	1.08	0.79	0.82	0.07	0.07
26	17 : 0	0.6851	17.00	0.39	0.03	0.05	0.04	0.09
27	16 : 2 (n-4)	0.7262	17.15	0.88	1.09	1.08	0.04	0.05
28	17 : 1 (n-8)	0.7574	17.26	0.21	0.36	0.05	0.09	0.08
29		0.7913	17.38	0.10	0.23	0.13	ND	0.03
30	*iso*-18 : 0	0.8314	17.51	0.02	0.10	0.10	ND	ND
31	16 : 4 (n-3)	0.8519	17.58	1.10	0.78	2.80	0.33	0.42
32		0.8866	17.68	ND	ND	0.01	ND	0.21
33		0.9050	17.74	0.01	ND	0.10	ND	ND
34		0.9628	17.90	ND	ND	ND	ND	0.05
35	18 : 0	1.000	18.00	3.36	1.85	0.47	0.41	1.69
36	18 : 1 (n-13)	1.044	18.12	0.10	0.09	ND	ND	0.61
37	18 : 1 (n-11)	1.081	18.21	0.14	0.92	ND	ND	ND
38	18 : 1 (n-9)	1.095	18.25	11.58	12.36	7 63	4.10	4.16
39	18 : 1 (n-7)	1.124	18.32	3.14	5.01	0.76	ND	3.57
40	18 : 1 (n-5)	1.165	18.42	0.13	0.17	0.46	ND	0.19
41		1.211	18.52	0.15	0.02	ND	ND	ND
42	18 : 2 (n-9)	1.245	18.60	ND	0.02	ND	0.04	1.01
43	18 : 2 (n-6)	1.288	18.69	0.92	0.83	0.66	3.93	1.81
44	18 : 3 (n-9)	1.317	18.75	ND	ND	ND	ND	0.14
45		1.368	18.85	0.34	0.22	0.15	ND	0.02
46	18 : 3 (n-6)	1.407	18.93	0.15	0.11	0.09	1.03	0.55
47	19 : 0	1.444	19.00	0.05	ND	ND	ND	ND
48		1.495	19.09	ND	ND	ND	ND	0.03
49	19 : 1 (n-8)	1.513	19.12	0.06	0.09	0.03	ND	0.01
50	18 : 3 (n-3)	1.583	19.25	0.78	0.62	0.43	13.75	0.94
51	18 : 4 (n-3)	1.734	19.49	2.08	2.12	3.63	38.91	1.74
52	18 : 4 (n-1)	1.774	19.55	0.05	0.39	0.42	ND	ND
53		1.822	19.63	0.01	ND	ND	ND	0.01
54		1.868	19.69	0.01	0.02	0.10	ND	0.03
55		1.994	19.87	ND	ND	0.05	ND	0.03
56	20 : 0	2.091	20.00	0.14	0.07	0.10	0.12	0.43
57	20 : 1 (n-15)	2.166	20.10	ND	ND	ND	ND	3.22
58	20 : 1 (n-11)	2.228	20.18	0.89	7.96	2.86	ND	0.58
59	20 : 1 (n-9)	2.265	20.22	1.56	3.67	2.22	ND	4.60

Table III. Continued.

Peak no.	Fatty acid	Retention data RRT	ECL	Composition (wt%) Sardine *Sardinops melanosticta* total lipids	Pollack *Theragra chalcogramma* total lipids	Zooplankton copepod[b] wax esters	Seaweed *Undaria pinnatifida* total lipids	Sea urchin *Strongylocentrotus intermedius* total lipids
60	20 : 1 (n-7)	2.356	20.31	0.19	0.10	0.05	ND	0.57
61	20 : 2 (*Δ*5, 11)	2.372	20.35	ND	ND	ND	ND	6.02
62	20 : 2 (*Δ*5, 13)	2.399	20.38	ND	ND	ND	ND	1.43
63		2.439	20.43	0.03	0.08	0.03	ND	ND
64	20 : 2 (n-9)	2.515	20.51	0.28	ND	ND	ND	0.59
65	20 : 3 (n-9)	2.592	20.59	0.11	0.03	ND	ND	1.04
66	20 : 2 (n-6)	2.668	20.67	0.11	0.12	0.04	ND	1.09
67		2.789	20.80	ND	ND	ND	ND	0.12
68	20 : 3 (n-6)	2.902	20.91	0.07	0.03	ND	0.22	0.97
69	20 : 4 (n-6)	3.037	21.03	1.19	0.32	0.15	7.27	7.95
70		3.153	21.14	ND	ND	ND	ND	0.45
71	20 : 3 (n-3)	3.273	21.24	0.05	0.05	ND	ND	0.45
72	20 : 4 (n-3)	3.566	21.48	0.55	0.67	0.42	0.44	0.69
73	20 : 5 (n-3)	3.780	21.64	13.72	11.59	12.74	13.57	4.15
74	22 : 0	4.365	22.00	0.09	ND	ND	ND	0.28
75	22 : 1 (n-11)	4.591	22.18	1.60	11.84	3.48	ND	ND
76	22 : 1 (n-9)	4.689	22.24	0.42	1.23	0.29	ND	2.14
77	22 : 1 (n-7)	4.849	22.34	0.06	0.21	ND	ND	0.03
78	22 : 2 (*Δ*7, 13)	4.978	22.43	ND	ND	ND	ND	0.11
79	22 : 2 (*Δ*7, 15)	5.095	22.54	ND	ND	ND	ND	0.54
80		5.373	22.64	ND	ND	ND	ND	0.15
81	21 : 5 (n-3)	5.542	22.72	0.46	0.46	0.31	ND	0.09
82	22 : 4 (n-6)	6.378	23.12	0.06	0.05	ND	ND	0.07
83	22 : 5 (n-6)	6.693	23.25	0.23	0.14	ND	ND	ND
84	22 : 5 (n-3)	7.855	23.71	1.52	1.11	0.59	ND	0.05
85	22 : 6 (n-3)	8.290	23.86	9.34	7.41	4.77	ND	0.07
86	24 : 1 (n-9)	9.657	24.30	0.42	0.71	ND	ND	ND

a) GC conditions: 67 m×0.28 mm ID glass WCOT SILAR-5CP column. Column temperature, 170°C. Carrier gas (N_2) flow rate, 1 ml/min. Inlet pressure, 1.0 kg/cm^2. Splitting ratio, 1/25. Sample size, 0.2~0.4 µl of a 1% (wt/vol) solution in hexane. RRT=retention time between the front of solvent deflection and the peak maximum (18 : 0=1.000). ECL=equivalent chain length.

b) Mixture of *Calanus cristatus* and *Calanus plumchrus*.

c) ND=not detected.

In the seaweed of Table III there is a relatively high proportion(7.27%) of 20:4ω6 (arachidonic acid). This is found in similar proportions in several North Atlantic seaweeds[64] as well as in certain (Rhodophyceae) unicellular phytoplankters[66]. In fish oils from these latitudes 20:4ω6 is 1% of total fatty acids (Table III, cf. redfish, Table II), and this is also true of the southerly latitudes of the Atlantic Ocean off South Africa[48]. In the Indian Ocean (Table IV, see also oil sardine, Table II) the level of 20:4ω6 in fish lipids ranges up to 6% or even 14% of total fatty acids[67,68] in some marine species. Recently we have a report[69] of the dietary influence in man of high levels of 20:4ω6 in fish from Australian waters. This gap in our knowledge has been both temporal and geographic and the details of marine lipids in the literature from latitude 45‡ may not be applicable within latitudes 0—20°N or S.

Table IV. Comparison of some nutritionally important fatty acids in fish from the Indian Ocean.

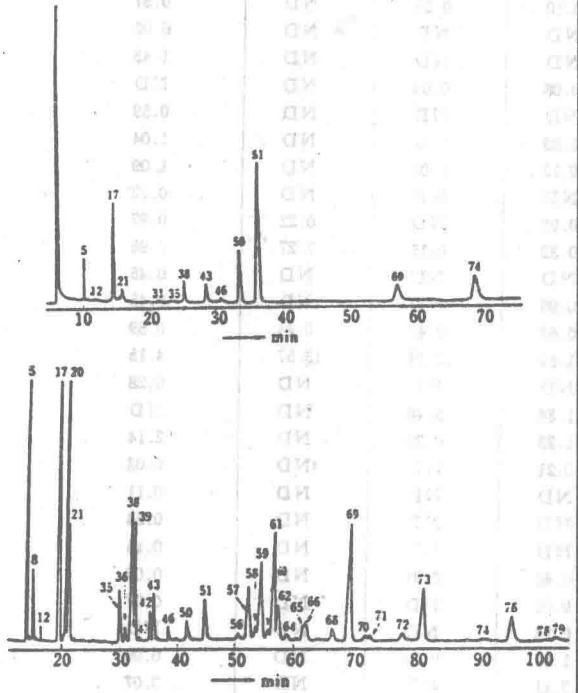
Akulin and Pervuninskaya 1978[68]

	Sphyrna zygaena	*Carcharhinus jonsoni*	*Alopias vulpinus*	*Carharys glanka*
% Lipid	0.2	0.6	0.3	0.5
18:2 ω6	0.5	1.2	1.3	0.1
20:4 ω6	11.0	14.2	5.9	6.6
20:5 ω3	2.1	2.0	0.8	2.6
22:6 ω3	17.8	7.8	12.9	16.0

Nair and Gopakumar 1978[67]

	Sphyraena langsar	*Psettodes erumei*	*Johnius argentatus*	*Lutianus waigiensis*
% Lipid	1.5	0.9	1.6	3.1
18:2 ω6	2.8	3.6	2.9	4.9
20:4 ω6	3.2	6.3	4.3	3.2
20:5 ω3	3.9	5.9	7.1	0.9
22:6 ω3	10.3	10.4	4.9	1.0

Figure 1. Comparison illustrating the simplicity of a fatty acid of an alga *Undaria pinnatifida* (above) with those of a sea urchin *Strongylocentrotus intermedius* (below). For peak identifications see *Table III.* Solvent peak not shown in sea urchin record. Note complexity of 20:1 region due to NMID (components 61 and 62). Analysis on glass WCOT column with SILAR-5CP liquid phase as described in *Table III.* Reproduced from Yukagaku 29:855-865 (1980) by permission of the journal and of Y. Itabashi and T. Takagi.



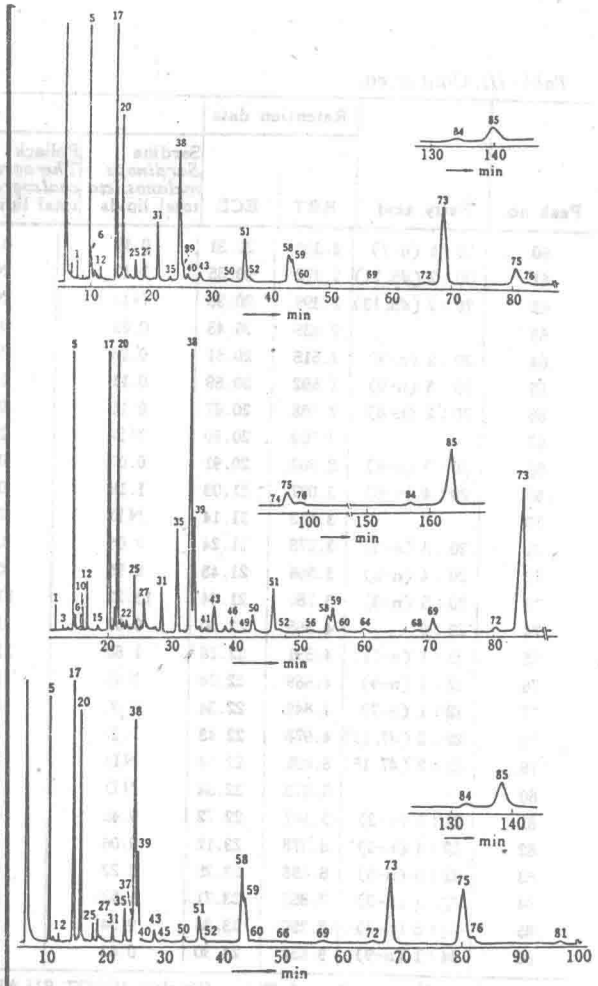


Figure 2. Comparison of three levels of complexity among fatty acids of three pelagic marine animals. Top: fatty acids from mixture of *Calanus cristatus* and *Calanus plumchrus*. Centre: fatty acids of oil from sardine *Sardinops melanosticta*. Bottom: fatty acids of lipid from Alaska pollack *Theragra chalcogramma*. Note low proportion of 18:1 ω7 (component 39) in copepod lipids and high proportion of 20:5ω3 and 22:6ω3 (components 73 and 85) in sardine oil. The Alaska pollack lipid has the highest proportion of 22:1ω 11 and 22:1ω 9 (components 75 and 76). Analyses on glass WCOT column with SILAR-5CP liquid-phase as described in *Table III.* Solvent peak omitted in oil sardine record. Reproduced from *Yukagaku 29*:855-856 (1980) by permission of the journal and of Y. Itabashi and T. Takagi.

As an example of inadvertent bias, we can select the fatty acids of molluscs as guides to fatty acid utilisation. These animals are economically important, easily raised, and highly visible to administrators. Therefore research is well supported. In Table V are compared the total *w*3 fatty acids (mostly 18:2*w*6 and 20:4*w*6) and the total *w*3 fatty acids (mostly 18:3*w*3, 18:4*w*3, 20:4*w*3, 20:5*w*3, 22:6*w*3) averaged for individual phytoplankter culture results from five laboratories (details in[20]). Clearly the ratio *w*6 : *w*3 is about 1 : 5. In the ocean quahog, the common mussel, and the scallop the ratio is about 1 : 15, while in the oysters it is about 1 : 4 or less. The conclusions of this examination of the status of *w*6 and *w*3 fatty acids were that molluscs took in dietary fatty acids and deposited them temporarily in triglycerides or used them for energy. Tables VI and VII show this deposition effect in *C. virginica* for both monoethylenic fatty acids and the six important polyunsaturated fatty acids. The fatty acids of the cellular phospholipids are drawn[12] from this temporary pool of fatty acids for the particular needs of the species (Tables VIII and IX).

It is important to note that although the capability for chain extension of 18:2*w*6 to 20:4*w*6, and of 18:3*w*3 to 20:5*w*3 (or 22:6*w*3) no doubt exists in these molluscs, it may not be required if preformed 20:4*w*6 and 22:6*w*3 are freely available from a mixed algal diet.

Molluscs from Australian waters contain 20:4*w*6 and 20:5*w*3 in roughly equal proportions[75]. Gastropods may browse on a particular algal growth and are unreliable for generalisations. Local Rhodophyta and Phaeophyta, but no Chlorophyta, contain very high levels of 20:4*w*6[76]. Filter feeders are preferred for food

Table V. Comparisons of total percentages of C_{18}-C_{20} $\Sigma\omega6$ and $\Sigma\omega3$ fatty acids averaged from marine unicellular algae of various types cultured by five different laboratories[20], with similar averages for phospholipids of individual species of molluscs (cf *Tables VI and VII*).

Type of fatty acid	Averaged Marine Algal Cultures				
$\Sigma\omega6$	6.0	5.8	6.5	5.7	5.2
$\Sigma\omega3$	30.1	32.5	12.4	34.1	26.3

Mollusc Phospholipids

Sample	Flesh	*Arctica islandica*[70] hepatopancreas	*Mytilus edulis*[71] body	*Placopectan maximus*[71] body
$\Sigma\omega6$	3.0	1.5	1.9	1.4
$\Sigma\omega3$	45.7	25.0	26.5	28.6

Sample	*Crassostrea virginica*[72] Hatchery-reared spat	*Crassostrea virginica*[73] body	*Ostrea edulis*[73] body
$\Sigma\omega6$	9.2	5.9	8.1
$\Sigma\omega3$	35.5	23.8	11.9

Table VI. Comparison of weight percentages of the important C_{16} and C_{18} monoethylenic fatty acids in total lipids of *Pavlova lutheri Tetraselmis suecica* and *Dunaliella tertiolecta* with triglyceride and phospholipid fatty acids of experimentally fed *Crassostrea gigas* spat from Langdon and Waldock 1981

	P. lutheri	*T. succica*	*D. tertiolecta*
16:1 ω9 (algae)	—	1.8	0.6
spat triglyceride	trace	2.0	0.3
spat phospholipid	trace[1]	0.4	0.7
18:1 ω9 (algae)	3.3	22.7	6.6
spat triglyceride	2.4	24.6	13.1
spat phospholipid	2.2[1]	3.8	3.2
20:1 ω11/ω9 (algae)	—	(2.85)[2]	—
spat triglyceride	6.5	5.6	0.9
spat phospholipid	2.6	3.7	1.5
16:1 ω7 (algae)	25.8	2.0	3.6
spat triglyceride	4.0	trace	2.2
spat phospholipid	2.2[1]	1.0	1.2
18:1 ω7 (algae)	2.5	trace	0.4
spat triglyceride	14.7	trace	0.3
spat phospholipid	7.2[1]	3.4	10.0
20:1 ω7 (algae)	—	(2.85)[2]	—
spat triglyceride	2.6	6.9	1.8
spat phospholipid	5.7	4.5	4.1

[1]Total lipid (29% triglyceride, 71% phospholipid) as phospholipid data not given.

[2]Total 20:1.

Table VII. Comparison of weight percentages of some C_{18}, C_{20}, and C_{22} fatty acids in total lipid of *Chaetoceros calcitrans, Pyramimonas virginica* and *Isochrysis galbana* with triglyceride fatty acids of experimentally fed *Crassostrea gigas* larvae (from Waldock and Nascimento 1979

	C. calcitrans	*P. virginica*	*I. galbana*
18:2 ω6 (algae)	0.2	2.6	4.3
(larvae)	0.7	1.5	6.5
18:3 ω3 (algae)	3.0	10.6	11.0
(larvae)	2.9	7.5	10.6
18:4 ω3 (algae)	0.5	4.4	9.2
(larvae)	1.2	3.9	8.9
20:4 ω6 (algae)	trace	—	0.2
(larvae)	0.5	0.5	0.8
20:5 ω3 (algae)	15.4	1.3	3.6
(larvae)	22.0	4.1	3.2
22:6 ω3 (algae)	1.9	13.7	18.9
(larvae)	1.6	11.8	18.4

Table VIII. *Percentages (w/w) of selected saturated and monoethylenic fatty acids of different lipid classes of some bivalve species. ND = not determined, PL = phospholipids, TG = triglycerides, SE = sterol esters.*

	Ackman et al. (1974)						Longdon and Waldock (1981)						Watanabe and Ackman (1974)				Gardner and Riley (1972)					
	Artica islandica						*Crassostrea gigas* spat						*Crassostrea virginica*		*Ostrea edulis*		*Mytilus edulis*			*Pecten maximus*		
	Flesh			Hepatopancreas																		
Fatty Acid	PL	TG	SE	PL	TG	SE	PL[1]	TG[1]	PL[2]	TG[2]	PL[3]	TG[3]	PL	TG	PL	TG	PL	TG	SE	PL	TG	SE
14:0	0.4	3.5	4.2	1.4	4.6	7.8	0.7	4.8	1.1	1.1	2.1	2.4	2.0	4.4	6.0	8.1	1.9	3.0	1.5	4.4	4.0	4.6
16:0	12.9	32.4	18.6	22.5	30.0	20.3	7.7	18.6	14.4	20.2	13.2	20.1	26.7	34.8	31.5	42.0	10.7	13.6	11.5	19.0	11.1	10.8
18:0	6.3	7.8	7.8	11.6	5.9	7.8	2.2	1.4	3.2	7.9	3.6	4.7	6.2	2.2	12.7	7.5	10.0	4.7	5.9	4.9	5.0	6.2
Total	19.6	43.8	30.6	35.5	29.6	35.9	10.6	24.8	19.0	29.2	20.9	27.2	34.9	41.4	50.2	57.6	22.6	21.3	18.9	27.4	20.1	21.6
16:1ω9	0.2	0.8	2.4	ND	0.5	4.7	trace	0.3	0.7	0.3	0.4	2.0	0.6	1.3	1.5	1.7	4.8	8.3	6.7	3.4	7.6	5.9
16:1ω7	1.7	12.6	2.7	1.2	14.4	4.4	0.5	0.7	1.2	2.2	1.0	trace	1.8	4.0	2.5	5.9						
18:1ω13	2.1	0.8	1.1	1.9	0.9	0.3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND						
18:1ω9	1.9	3.7	6.9	3.2	2.7	9.8	5.9	10.0	3.2	13.1	3.8	24.6	3.7	4.8	2.7	5.4	6.8	7.4	9.2	4.8	7.8	5.6
18:1ω7	2.9	8.3	5.3	5.2	10.5	3.9	0.6	0.8	10.0	0.3	3.4	trace	3.1	5.6	1.3	5.9						
20:1ω11	0.3	0.4	0.8	0.3	0.4	0.5	1.6	4.8	1.5	0.9	3.7	5.6	1.9	0.8	1.0	0.6						
20:1ω9	1.1	1.7	4.0	1.6	1.6	3.2							1.5	0.5	0.8	0.4	6.2[4]	8.8[4]	6.1[4]	3.3[4]	2.6[4]	3.8[4]
20:1ω7	1.3	3.3	18.8	2.6	3.4	6.4	6.2	6.1	4.1	1.8	4.3	6.2	4.2	1.3	2.2	3.4						
Total	11.7	33.6	42.0	16.0	34.4	32.2	15.5	22.7	20.7	18.6	16.8	39.1	17.5	18.5	12.0	22.3	17.8	24.3	22.0	11.5	18.0	15.3

1. Hatchery reared (cultured in coarse-filtered seawater supplemented with mixed algal diet.
2. Reared on *Dunaliella tertiolecta* (Butcher).
3. Reared on *Tetraselmis suecica* (Butcher).
4. May contain NMID.

Table IX. *Percentages (w/w) of selected polyunsaturated fatty acids of different lipid classes of some bivalve species. NMID refers to "non-methylene-interrupted" diethylenic fatty acids. ND = not determined, PL = phospholipids, TG = triglycerides, SE = sterol esters.*

	Ackman et al. (1974)						Longdon and Waldock (1981)						Watanabe and Ackman (1974)				Gardner and Riley (1972)					
	Artica islandica						*Crassostrea gigas* spat						*Crassostrea virginica*		*Ostrea edulis*		*Mytilus edulis*			*Pecten maximus*		
	Flesh			Hepatopancreas																		
Fatty Acid	PL	TG	SE	PL	TG	SE	PL[1]	TG[1]	PL[2]	TG[2]	PL[3]	TG[3]	PL	TG	PL	TG	PL	TG	SE	PL	TG	SE
18:2ω6	0.3	0.4	0.9	0.5	1.0	0.1	trace	0.3	0.5	1.4	0.2	trace	0.9	2.6	0.9	1.3	1.3	2.7	2.0	0.7	1.6	1.6
18:3ω3	1.3	1.3	0.3	2.1	0.8	1.5	5.3	1.9	9.8	15.6	3.8	7.9	3.8	2.9	4.9	1.1	2.2	0.3	2.5	0.9	0.1	trace
18:4ω3	ND	0.7	0.3	0.4	2.8	0.9	0.4	trace	0.6	trace	1.4	1.4	1.1	3.0	1.0	0.6	4.8	6.1	4.4	3.2	5.8	4.6
20:4ω6	2.1	0.2	ND	0.8	0.3	0.3	6.3	3.1	6.3	2.3	5.6	1.8	4.6	1.1	2.6	0.5	0.6	-	trace	-	-	1.6
20:4ω3	2.9	ND	ND	3.5	0.2	1.4	0.8	1.0	1.4	1.0	0.5	trace	0.1	0.6	trace	0.1	4.2	4.2	4.1	1.4	1.1	2.7
20:5ω3	15.3	5.5	0.8	9.6	20.1	0.9	11.4	7.0	4.2	1.1	11.2	5.4	8.1	9.1	6.9	2.2	9.6	12.9	7.9	12.2	9.5	2.4
22:4ω6	0.6	ND	ND	0.1	ND	ND	0.5	0.5	ND	trace	0.8	ND	0.1	trace	1.1	ND	-	-	trace	0.7	-	trace
22:5ω6[4]	0.1	ND	ND	0.1	ND	ND	2.4	2.2	1.9	1.7	1.2	0.5	0.3	0.1	trace	ND	-	-	-	-	-	-
22:5ω3	2.2	0.4	ND	1.0	ND	1.0	1.8	0.5	trace	trace	2.2	ND	0.4	0.1	0.3	trace	2.5	1.5	trace	2.5	1.5	trace
22:6ω3	23.2	2.8	ND	8.4	ND	9.8	15.8	10.5	7.4	1.7	4.7	0.5	10.3	4.7	2.3	0.9	3.2	3.8	3.2	8.4	7.9	1.6
Total	48.7	11.3	2.3	26.5	25.2	6.8	44.7	27.0	32.1	25.0	31.6	17.5	29.7	24.2	20 0	6.7	28.4	33.5	24.1	30.0	27.5	14.5
NMID	11.5	3.4	10.3	10.1	2.1	3.2	ND	ND	ND	ND	ND	ND	7.4	3.0	ND	ND	ND	ND	ND	ND	ND	ND

1. Hatchery reared (cultured in coarse-filtered seawater supplemented with a mixed algal diet.)
2. Reared on *Dunaliella tertiolecta* (Butcher).
3. Reared on *Tetraselmis suecica* (Butcher).
4. Data reported by Gardner and Riley (1972) is here reported as 22:5ω3.

web hypotheses but one must be alert to the presence of zooxanthellae in animals. In latitudes 14—18° clams contained only traces of 20:4ω6, whereas corals had 2.3—7.8% in their fatty acids[63].

The fresh fish purchased in Sydney by Pearson[77] do not show more than moderate amounts of 20:4ω6, and the New Zealand shellfish and fish examined in a similar study by local scientists[78,79] have at most 4.2% of 20:4ω6.

The Indian Ocean results summarized in Table IV, and the recent fish analyses of O'Dea and Sinclair[69] from the Australian latitude 17°, strongly suggest that sponges and corals in the latitudes 0—20° are the basic sources of high levels of 20:4ω6 relative to 20:5ω3. Data for marine invertebrates have recently been assembled and reviewed[2,3], but such data for corals can be unreliable. Thus a Caribbean coral *Mancina areolata* is reported to contain *no* polyunsaturated fatty acids[80]. In a gorgonian of similar origin examined in my laboratory we recovered fatty acids with 4.1% 20:4ω6, 0.5% 20:5ω3 and 2.8% 22:6ω3, although the sample was by no means fresh and the published results[81] erroneously showed 20:4ω6 as 20:1!.

We must accept that the food chain, or rather food web, in the latitudes 0—20° differs appreciably from that of more northerly latitudes[82]. Copepods and similar crustacea are probably less important as selective converters of plant fatty acids than in the northern waters, their place being taken by corals, gorgonians, sponges, and similar invertebrates in the shallower waters[83]. It is not altogether surprising that the function of arachidonic acid in these animals has led to profitable investigations for prostaglandins[84].

In this energy transport from phytoplankters to fish the wax esters can play an important role in shallower waters[83]. The vertical distribution of invertebrates and fish in deeper tropical waters[85] may be more akin to the direct energy transfer in shallower northern waters[86], but the occurrance of wax esters in various types of organisms is probably a case of convergent evolution and not a matter of food web transfer.

In this short review it has been impossible to include the fascinating new ideas of dietary 20:5ω3 as a potentially useful agent in preventing cardiovascular disease[87-89]. Almost the only practical source of this fatty acid is fish lipid or fish oils[21,48,90,91]. By a curious quirk it is also reported that platelet aggregating

factor or PAF (acetyl glyceryl ether phosphoryl choline) can be most potent when made from the natural glyceryl ethers present in the liver oil of the ratfish *Hydrolagus colliei*[92]. It is also worth recording that methoxy-substituted glyceryl ethers[93] may inhibit tumour growth[94]. Thus, both these substituted or regular glyceryl ethers[82,95,97] found in elasmobranchs and some other species are not only of general interest to lipid chemists but may also be valuable raw materials for future pharmaceutical industries.

We have just begun to fully understand the nutritional needs of fish in terms of fatty acids[98], and perhaps better understand some aspects of those of molluscs[20,63,71]. The origin of the unusual 22:1ω11 alcohol of copepod wax esters and its role in fish nutrition are yielding to advanced biochemical studies[99,100]. There is a need to develop in tropic and semitropic regions parallels to the marine lipid work long carried out in northerly latitudes. This conference will, I hope, have a stimulating effect on this process.

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