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ESSENTIAL FATTY ACID CONTENT OF FEEDSTOCKS

USED IN AQUACULTURE

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ESSENTIAL FATTY ACID CONTENT OF FEEDSTOCKS USED IN AQUACULTURE

Summary

One of the most important steps in aquaculture is the successful rearing of animals from eggs through to larval and juvenile stages. Animals at this stage are particularly vulnerable to environmental conditions and to any deficiencies in their diet. Microalgae are widely used as a live feedstocks for the rearing of all growth stages of molluscs, for the larval stages of fish and crustaceans and for zooplankton species which are also used as live feeds.

The aquaculturist has many hundreds of microalgal species to choose from, but these vary widely in their nutritional properties, growth characteristics and palatability. Our research has been designed to identify specific microalgal species which promote the successful rearing of marine animals, and to formulate general principles concerning nutritional characteristics of the different algal classes.

Research has established that certain polyunsaturated fatty acids (PUFA) must be present in the diet of marine animals for good growth and survival. This is particularly important during the early growth stages where the animal may have limited capacity to synthesize particular essential fatty acids and other compounds.

We have carried out extensive studies of the fatty acids and lipid compositions of microalgae from all of the major algal classes used in mariculture. This work has established that chlorophytes (green algae) are very poor sources of essential PUFA. This is contrary to earlier views which were based on the incorrect classification of "marine *Chlorella*" as a green alga. Our data confirm that this alga is in fact a eustigmatophyte, and we have now demonstrated that species of *Nannochloropsis* from this class of microalgae are an excellent source of 20:5(n–3) PUFA. In contrast, green algae from the class Prasinophyceae contain only small amounts of PUFA and in most cases they are best used as components of a mixed microalgal diet.

We have demonstrated that prymnesiophytes are excellent sources of PUFA. Species of *Isochrysis* contain 20:5(n–3), but not 22:6(n–3), whereas *Pavlova* species contain high concentrations of both fatty acids which explains their value as live feeds. Several tropical Australian isolates of *Pavlova* spp. have been identified which should prove to be useful feeds in tropical hatcheries. Other good sources of 20:5(n–3) PUFA include diatoms, cryptomonads and rhodophytes.

Other lipid classes may also be important in animal nutrition, but much more work needs to be done in this area. For example, crustaceans have an absolute requirement for sterols in their diet which they modify to produce cholesterol. The sterol compositions of microalgae vary dramatically, although some systematic characteristics for the various classes are emerging. One intriguing result is the identification of cholesterol as the major sterol in eustigmatophytes. When these algae are used as feedstocks the animal is able to obtain its sterol requirements already pre–formed.

Several of the algae were found to contain some very unusual lipids. These are apparently not toxic, but whether they have nutritional value is unknown. In the case of very long–chain unsaturated ketones (alkenones) found in *Isochrysis*, it appears that the animals are unable to metabolise the compounds and excrete them unchanged. This enhances the production of pseudofaeces which can lead to increased fouling of the tanks.

The fatty acid and lipid composition of microalgae can be influenced by the choice of culture conditions. However, these effects mainly change the amounts of lipids per cell rather than causing a dramatic shift in the proportions of the various fatty acids. Our experiments show that changes in light intensity do not produce major changes in lipid composition, but the stage at which an alga is harvested can be an important determinant of lipid composition and content. Triacylglycerols often accumulate during stationary phase growth, and since these compounds provide a readily utilised energy store this may increase their dietary value to marine animals. Although specific responses vary from one class of microalgae to another, there is an opportunity for the mariculturist to optimize the nutritional value of microalgae from a careful choice of growth conditions and harvesting strategy.

Zooplankton such as rotifers (*Brachionus*) and brine shrimp (*Artemia*) are widely used as intermediate food organisms in mariculture. Ours studies show that rotifers rapidly take up lipids from their microalgal diets and they are an excellent means of transferring essential fatty acids to the larval animal. Within just 3 hours, the fatty acids of rotifers resembles very closely the composition found in the microalgal diet. However, the content of 22:6(n–3) PUFA in brine shrimp remains quite low irrespective of the content of this fatty acid in its algal or artificial diet. This provides one reason why *Artemia* is not an optimum feedstock in some applications.

Objectives as set out in original proposal:

(1) Use a quick screening method to measure 20:5(n–3) and 22:6(n–3) fatty acids in microalgae from various Australian hatcheries.

(2) Compare these values with data already obtained from algae cultured under standard conditions. If not the same, identify features of the hatchery operation that could be changed so as to increase the nutritional value of the microalgal food.

(3) In conjunction with FIRTA Grant 86/81, determine from controlled laboratory experiments how to optimize the content of essential fatty acids by changes in culture conditions that are relevant to hatchery production.

(4) With FIRTA grant 86/81, to conduct animal growth trials (oysters and prawns) to assess the impact of improved fatty acid content on animal production.

(5) Repeat of (1) above during the later stages of the project in order to determine the effect of suggested changes in hatchery operation upon nutritional value of the microalgal food.

Research undertaken as part of FIRTA grant 88/69 finished on December 31, 1991. Information on research achievements and progress made was included in our renewal proposal entitled *"Polyunsaturated fatty acid content and nutritional quality of aquaculture feedstocks"* (FRDC Grant 91/59). A more detailed account of the research is provided in this final research report.

STAFF INVOLVED ON PROJECT

Dr John K. Volkman	B.Sc.(Hons) Ph.D.	20%
Dr Peter D. Nichols	B.Sc.(Hons) Ph.D.	10%
Dr Shirley W. Jeffrey	B.Sc. M.Sc. Ph.D.	10%
Dr Christian D. Garland	B.Sc.(Hons) Ph.D.	5%
Mr Graeme A. Dunstan	B.Sc.	100%
Ms Stephanie M. Barrett	B.Sc.(Hons)	20%

Rationale for the Research

When our work commenced in 1988, there was a small but convincing literature showing that certain essential polyunsaturated fatty acids (also known by the acronyms PUFA or HUFA) such as eicosapentaenoic acid [20:5(n–3)] and docosahexaenoic acid [22:6(n–3)], must be present in the diet of marine animals to ensure their good growth and survival. The reason for this is that many animals cannot synthesize, or have a limited ability to synthesize, specific compounds needed for growth, and must obtain them from their diet. The source of PUFA used by the mariculture industry can be microalgae, prey species (copepods, brine–shrimps, rotifers) or artificial foods (pellets, capsules).

Our work was designed to study the fatty acid composition of different microalgae used as living feedstocks and identify those which had high contents of these essential fatty acids. Our intention was to identify the most appropriate feeds that would maximise animal production and minimize losses. Our analytical facility has provided fatty acid and lipid analyses and a source of technical information directed specifically to the needs of the aquaculture industry.

Good nutrition is particularly important at the larval and juvenile stages of the animal's growth since energy reserves are very low. It was also recognized that different animals have varying requirements for dietary lipids, proteins and carbohydrates. Another goal of our research was to establish the value of intermediate zooplankton such as brine shrimps (*Artemia*) and rotifers (*Brachionus*) as means of transferring essential nutrients to larval and juvenile animals.

Background

Biochemical studies of aquaculture feedstocks were carried out in close association with research funded by FIRTA grant 86/81 to S. W. Jeffrey and C. D. Garland entitled "*Microalgae for Mariculture*".

Cultures of microalgae were provided by Dr S. W. Jeffrey from the CSIRO Algal Culture Collection which has over 300 species in culture including over 50 Australian isolates. Ms J. M. Leroi (funded by FIRTA grant 86/81) cultured the microalgae and Dr Malcolm Brown (also funded by FIRTA grant 86/81) carried out concurrent analyses of amino acids and sugars thus providing a very detailed picture of the biochemical composition of the microalgal feeds.

Lipid and fatty acid analyses are carried out by the FIRTA–funded research projects scientist Mr Graeme Dunstan under the supervision of Drs J. Volkman and P. Nichols in the organic chemistry laboratories of the CSIRO Division of Oceanography. Additional assistance was provided by Miss Stephanie Barrett and other members of the research group as required.

The concentration of major lipid classes (hydrocarbons, free fatty acids, triacylglycerols, sterols and polar lipids) were determined with an Iatroscan Mk III TH-10 TLC-FID analyser (Iatron Laboratories, Japan). Total fatty acids were analysed as methyl esters produced by direct acid-catalysed transesterification of the lipid extract. This quick screening method avoided the time consuming task of saponifying the samples to remove non-saponifable lipids, followed by extraction of the total fatty acids and then esterification for analysis.

Lipid samples were analysed using capillary gas chromatographs equipped with flame ionisation detectors and cooled on-column injectors. Both nonpolar methyl silicone (BP-1) fused-silica and polar carbowax (BP-20) capillary columns were used to ensure correct identification and quantification of the many fatty acid isomers present.

Selected samples were also analysed by gas chromatography-mass spectrometry (GC-MS) as required to confirm the chemical structures of the compounds isolated. Double bond isomers of fatty acids were derivatised to DMDS adducts and analysed by GC-MS to confirm the positions of the double bonds

Results from the Project

Success was achieved in all of the major objectives. Full details are provided below. We analysed the lipids and fatty acid content of 31 strains of microalgae (see Appendix 1 for a full list), and identified several new species which should be very useful in tropical hatcheries. Feeding experiments with rotifers showed that these small zooplankton, which are used as intermediate foods for larval fish, take up dietary fats within a few hours.

These data have been made widely available to the fishing and mariculture industries through the "Microalgae for Mariculture" newsletters, conference presentations, workshops and scientific publications. A listing of papers, presentations and articles arising from our research is given in the Appendices.

Survey of fatty acids in microalgae

Detailed studies of the biochemistry of microalgae from all of the major classes used in mariculture were carried out. Our initial survey of the total fatty acids of the 10 species of microalgae most used by the aquaculture industry in Australia was published in 1989 (Volkman *et al.*, 1989). This work showed that there is a wide variation in the content of essential fatty acids in the various algal species (Fig. 1).

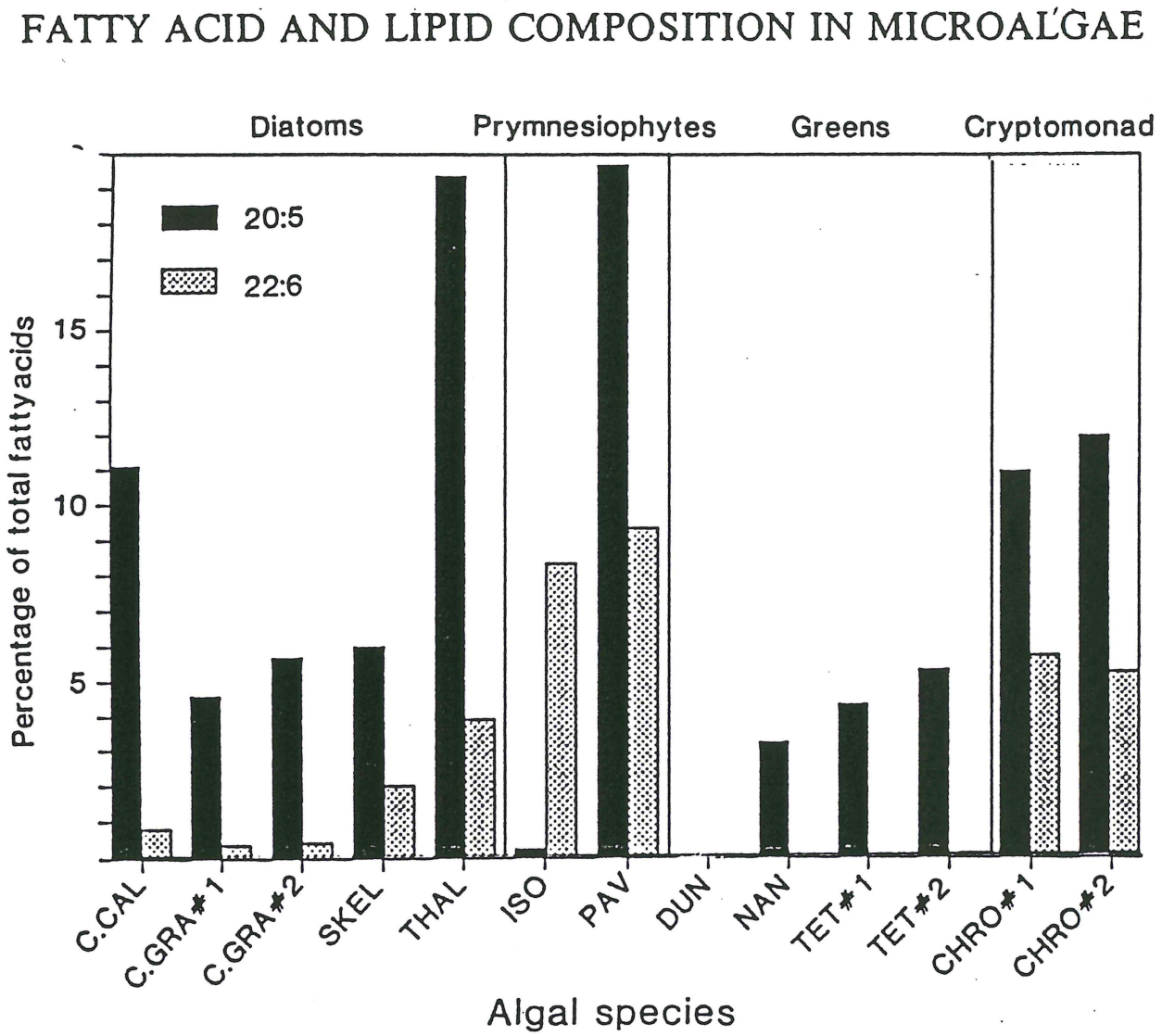

Figure 1. Concentrations of essential polyunsaturated fatty acids (as % of total fatty acids) in the 10 microalgae most commonly used as aquaculture feeds. Reproduced from Volkman *et al.* (1989).

In 1990 and 1991 we extended this work and conducted major surveys of the lipids in green algae (Chlorophyceae and Prasinophyceae), diatoms (Bacillariophyceae), golden-brown flagellates (Prymnesiophyceae) and the Eustigmatophyceae. A sufficient number of species was analysed to allow us to define those biochemical features which are characteristic of the various classes (Fig. 2). With these data, it is now possible to choose appropriate algae as possible feedstocks without the necessity of conducting animal growth trials for each species. For example, diatoms, eustigmatophytes and prymnesiophytes are good sources of the essential fatty acids 20:5(n-3), but of these microalgal classes only prymnesiophytes are also good sources of 22:6(n-3).

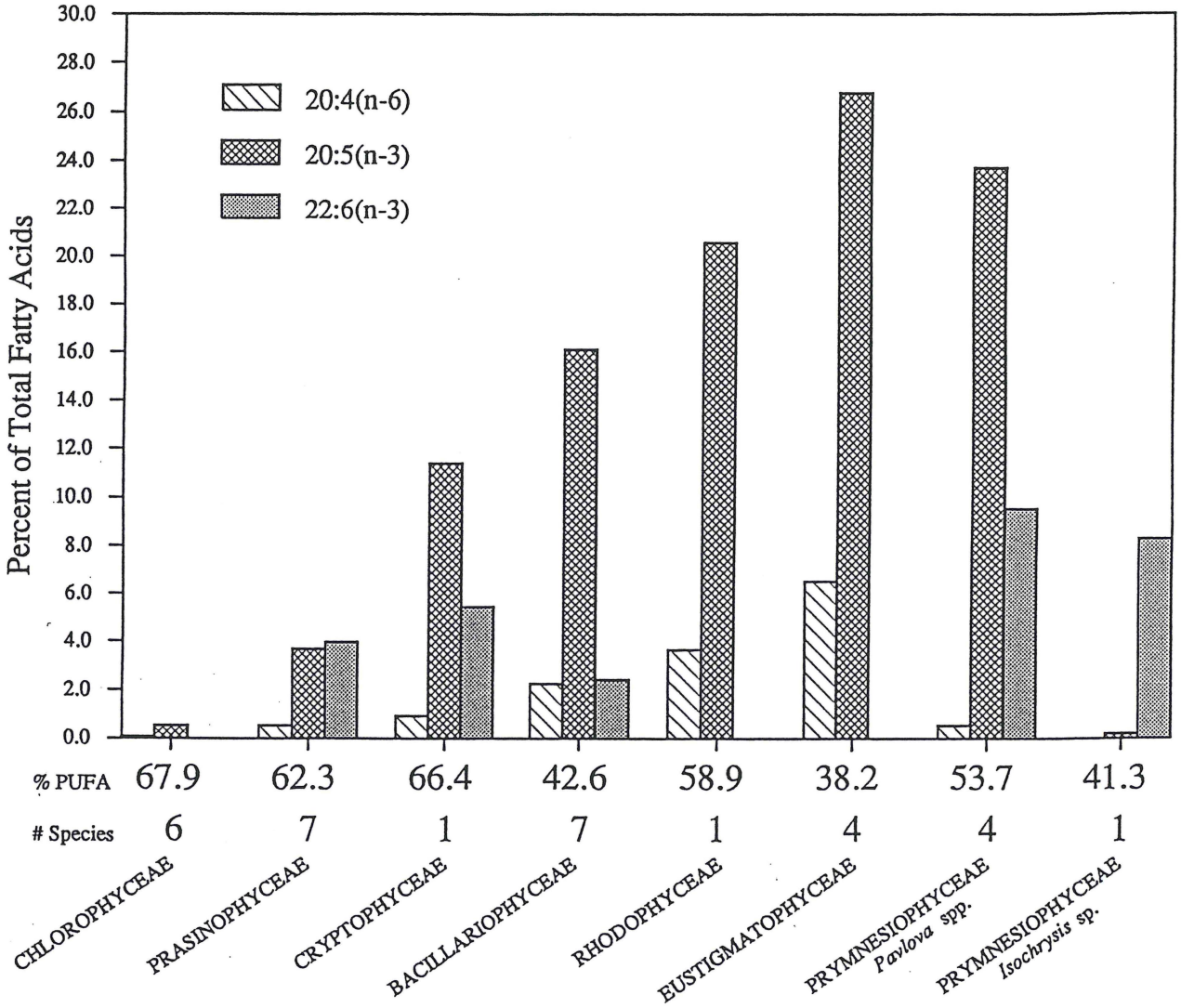

Figure 2. Concentrations of essential polyunsaturated fatty acids (as % of total fatty acids) in different microalgal classes. The data represent averages based on analyses of several species from each class. Reproduced from Volkman *et al.* (1992).

(i) *Fatty acids in green microalgae*

Thirteen species of green algae were studied. This work confirmed that very few species from the Chlorophyceae contain the essential C_{20} and C_{22} polyunsaturated fatty acids, and that their fatty acid distributions are dominated by C_{16} and C_{18} PUFA. However, all the prasinophytes contained significant amounts of 20:5(n–3) or 22:6(n–3), but not both (Fig. 3). These data provide an explanation why some green algae are poor feedstocks for larval production. However, the total fatty acid content of these algae can be high so that a mixed diet containing both green algae and other species rich in essential fatty acids may often be very successful. A paper describing these results, which also includes a major review of the fatty acids in green algae, was published (Dunstan *et al.*, 1992).

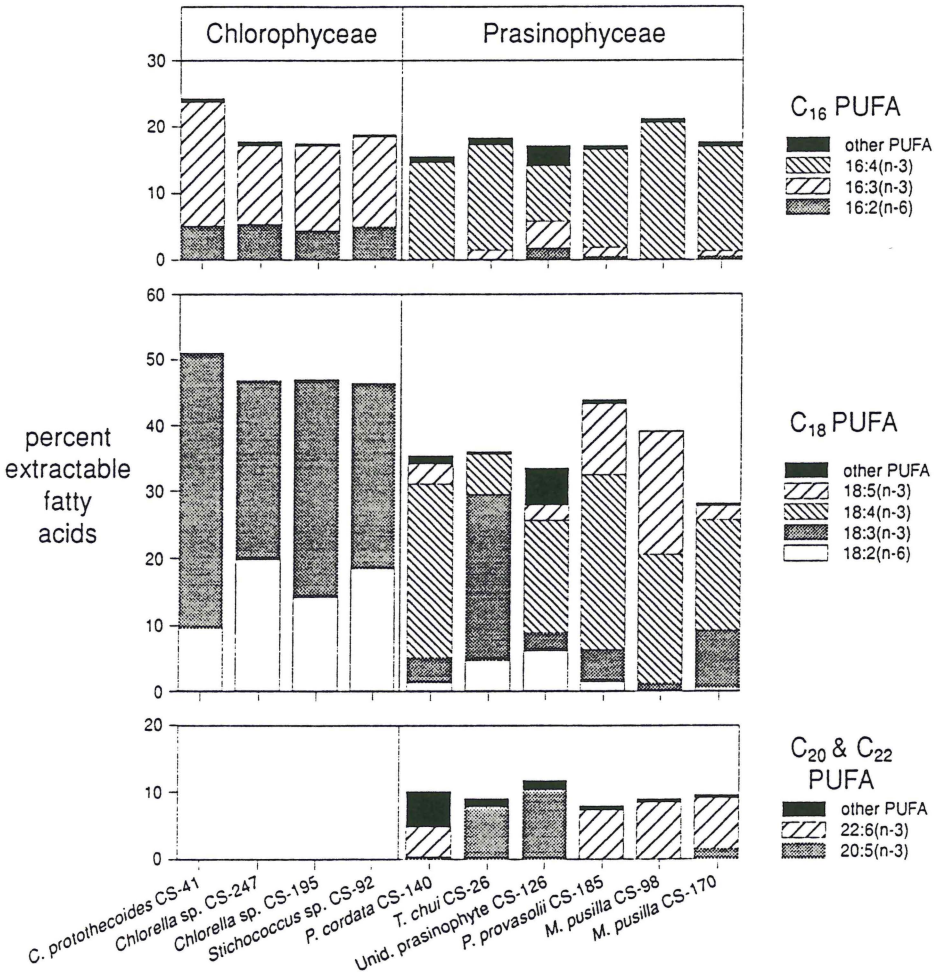

Figure 3. Percentage composition of major fatty acids in green algal from the classes Chlorophyceae and Prasinophyceae. Reproduced from Dunstan *et al.* (1992)

(ii) *Fatty acids and lipids in Pavlova species*

Prymnesiophyte algae have been used very successfully as mariculture feedstocks. The fatty acid content of four species of the prymnesiophyte genus *Pavlova* were determined (Table 1). *Pavlova salina* was shown to have a similar fatty acid profile to the widely used *Pavlova lutheri*, and because it has a better tolerance for higher temperatures should be well suited for use in tropical hatcheries. Two unnamed species also had very similar contents and distributions of fatty acids. The major fatty acids were 16:0, 16:1(n–7), 20:5(n–3) and 22:6(n–3). A paper discussing these results was published (Volkman *et al.*, 1991a).

Table 1. Percentage composition of major fatty acids in *Pavlova* species.

Species	*P. salina*	*P. salina*	*P. salina*	*Pavlova* sp.	*Pavlova* sp.	*Pavlova* sp.	*Pavlova* sp.	*P. lutheri*	*P. lutheri*
Strain	CS-49	CS-49	CS-49	CS-50	CS-50	CS-63	CS-63	CS-182	CS-182
Temp. (°)	27	20	20	20	20	20	20	20	20
Sample	A	A	B	A	B	A	B	B	*
Saturated fatty acids									
14:0	17.8	16.0	14.0	16.5	19.0	22.1	22.8	13.4	11.9
15:0	0.5	0.3	0.3	0.4	0.3	0.3	0.2	0.4	0.5
16:0	18.5	14.1	16.3	13.1	12.5	11.3	11.9	17.7	22.0
18:0	0.2	0.1	0.2	0.6	0.3	0.3	0.3	0.7	1.3
Subtotal	37.1	30.5	30.8	30.6	32.1	34.1	35.2	32.2	35.8
Monounsaturated fatty acids									
16:1 (n-7)	4.3	5.4	5.2	10.8	9.2	10.1	10.2	14.4	17.4
16:1 (n-5)	0.1	0.1	0.1	2.5	2.7	3.5	3.3	—	tr
16:1 (n-13) *t*	nd	nd	0.1	nd	0.4	nd	tr	0.2	—
18:1 (n-9)	0.7	0.2	0.4	0.7	0.4	0.5	0.4	1.6	1.8
18:1 (n-7)	tr	tr	—	0.2	0.3	0.1	0.2	1.2	1.4
20:1	tr	tr	tr	tr	tr	tr	0.1	tr	0.2
Subtotal	5.2	5.8	5.8	14.2	13.0	14.2	14.1	17.4	20.8
Polyunsaturated fatty acids									
16:2 (n-7)	0.2	0.1	—	0.1	—	0.1	–	0.3	0.2
16:2 (n-4)	0.3	0.3	0.8	0.1	1.7	0.1	1.2	0.5	0.2
16:3 (n-4)	0.2	0.2	0.1	0.4	0.3	0.3	0.2	0.3	0.4
18:2 (n-9)	tr	tr	tr	tr	tr	—	tr	0.3	0.4
18:2 (n-6)	2.0	1.8	1.9	1.1	1.0	1.8	1.5	2.3	1.6
18:3 (n-6)	2.4	0.7	1.2	0.5	0.4	0.5	0.6	1.1	0.4
18:3 (n-3)	1.1	1.3	1.4	2.0	1.4	1.4	1.1	1.6	1.9
18:4 (n-3)	10.7	15.2	13.6	11.2	11.0	9.3	9.5	7.5	6.2
20:4 (n-6)	0.7	0.4	0.8	0.6	0.7	0.6	0.6	0.6	tr
20:5 (n-3)	25.4	28.2	25.8	25.0	23.5	24.2	21.5	22.4	20.4
22:5 (n-6)	3.8	4.5	7.4	4.8	6.4	4.2	6.9	2.7	2.1
22:6 (n-3)	11.0	10.9	10.2	9.2	8.4	9.3	7.7	10.7	9.7
Subtotal	57.8	63.7	63.3	55.2	54.9	51.7	50.7	50.4	43.4
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Concentration (pg cell^{-1})	nd	nd	2.7	nd	2.6	nd	2.6	3.0	3.2

nd, not determined; tr, trace.

These algae were also found to contain unusual 4–methyl sterols and dihydroxy sterols which had not previously been found in any other species of microalgae (Volkman *et al.*, 1990). The nutritional effects of these compounds in the diet of animals is not known.

(iii) *Fatty acids and lipids in diatoms*

The lipids and fatty acids of 7 diatoms were studied. These included species already in use by the industry, plus a new species of *Skeletonema* that could be a useful food for prawns in tropical farms. Several benthic species that have possibilities as food for abalone were also examined. All of the diatoms had high concentrations of 20:5(n–3) (18–34%) (Fig. 1), indicating that they are worth testing as feedstocks although their low content of 22:6(n–3) (2–4%) may be a limitation for some feeding applications.

(iv) *Fatty acids of the Eustigmatophyceae*

An alga previously known as *Chlorella* (Japanese strain), which is widely used in mariculture operations, was analysed and shown to contain an unusually high concentration of 20:5(n–3). It is now known that this alga is a species of the Eustigmatophyceae (*Nannochloropsis oculata*). This has undoubtedly caused confusion in the mariculture industry and led to a mistaken belief that species of *Chlorella* (a green alga) are a good source of essential fatty acids.

Table 2. Percentage compositions of major fatty acids in eustigmatophytes

	N. salina CS-190 (20° C, A)	*N. oculata* CS-216 (20° C, A)	*N. oculata* CS-179 (20° C, A)	CS-246 (27° C, A[a])	Unnamed sp. CS-246 (27° C, A[a])	CS-246 (27° C, B)
Saturated fatty acids						
14:0	5.0	3.3	4.6	5.4	5.1	5.5
15:0	0.5	0.4	0.5	0.6	0.3	0.4
16:0	27.8	17.8	14.2	20.1	21.6	27.0
18:0	1.0	0.9	0.6	0.6	0.5	0.8
Subtotal	34.5	22.4	20.0	26.8	27.5	33.7
Monounsaturated fatty acids						
16:1(n-9)	0.1	—[b]	0.1	tr	—	—
16:1(n-7)	31.8	26.6	29.4	20.9	22.1	23.3
16:1(n-5)	0.4	0.2	0.2	0.1	0.1	0.1
16:1(n-13)*t*	0.1	0.3	0.4	0.5	0.8	0.9
17:1(n-8)	0.2	0.7	0.8	0.3	0.1	0.1
18:1(n-9)	8.3	7.7	6.3	4.6	2.2	2.3
18:1(n-7)	0.2	0.9	0.3	0.5	0.6	0.7
Subtotal	41.1	36.4	37.4	26.9	25.9	27.5
Polyunsaturated fatty acids						
16:2(n-7)	0.3	0.4	0.8	0.7	0.8	0.6
16:2(n-4)	0.1	0.1	0.1	0.2	0.4	0.4
16:3(n-4)	0.1	0.1	0.2	tr	0.2	0.1
18:2(n-9)	0.2	0.2	0.3	0.2	tr	tr
18:2(n-6)	1.5	2.9	2.0	2.7	2.3	2.2
18:3(n-6)	0.4	0.3	0.3	0.8	0.9	0.8
18:3(n-3)	0.2	0.4	0.1	0.9	0.1	0.1
20:3(n-6)	0.9	0.2	0.4	0.1	0.3	0.3
20:3(n-3)	tr	0.1	0.1	0.2	0.3	0.3
20:4(n-6)	4.0	7.1	8.8	6.4	5.9	5.1
20:5(n-3)	16.1	28.4	28.8	33.1	34.4	28.0
22:6(n-3)	—	—	—	—	—	—
Subtotal	24.2	40.8	42.2	45.7	46.0	38.2
Others	0.5	0.9	0.8	0.9	0.9	0.9
Concentrations						
pg fatty acid·cell^{-1}	0.93	0.53	0.27	0.39	0.44	0.34
mg fatty acid·g^{-1} dry wt	112	82	88	—	83	64

[a] Replicate cultures grown 3 months apart.
[b] Indicates that the fatty acid was not detected (<0.05% of total fatty acids).

Analysis of *Nannochloropsis salina,* and an unidentified Australian isolate thought to be closely related to *N. oculata,* showed that both contained high concentrations of 16:0, 16:1(n–7) and 20:5(n–3) (Table 2). Our work has confirmed that very high contents of 20:5(n–3) are typical of eustigmatophytes (Table 2 and Volkman *et al.*, 1991c) and that they should prove to be excellent food sources, particularly for use in tropical hatcheries.

The major sterol in eustigmatophytes was identified as cholesterol (Table 3). This may be an important dietary factor for animals such as crustaceans which have a limited capacity to synthesize cholesterol and must modify sterols obtained from the diet. As part of this research we also noted the presence of unusual polar lipids which contained C_{30}–C_{32} long–chain alcohols and diols (Volkman *et al.*, 1990). These compounds are common in many marine environments but a biological source had not been identified until our research was carried out.

Table 3. Concentrations of sterols and unusual lipids (femtogram per cell) in eustigmatophytes

	N. oculata CS-216		*N. oculata* CS-179		Unidentified sp. CS-246		*N. salina* CS-190	
Compound	Acid*	Base*	Acid	Base	Acid	Base	Acid	Base
Sterols								
Cholest-5-en-3β-ol	36.7	34.1	18.4	15.4	23.0	21.5	24.8	26.6
24-Ethylcholesta-5,24(28)E-dien-3β-ol	1.8	4.1	—	1.4	—	2.1	1.0	1.4
24-Ethylcholesta-5,24(28)Z-dien-3β-ol	1.0	4.8	—	1.7	—	2.5	0.3	6.6
24-Ethylcholest-5-en-3β-ol	—	—	—	—	—	—	7.0	7.5
Total sterols (fg/cell)	39.5	43.0	18.4	18.5	23.0	26.1	33.1	42.1
Long-chain alcohols								
$C_{30:1}$ *n*-alkenol	6.0	1.7	3.4	0.8	1.8	0.9	0.5	1.3
$C_{31:1}$ *n*-alkenol	0.6	tr	tr	tr	tr	—	0.9	tr
$C_{32:2}$ *n*-alkenol	4.3	tr	4.9	tr	0.7	tr	5.5	tr
$C_{32:1}$ *n*-alkenol	9.6	1.2	4.5	0.3	9.4	0.5	9.5	1.3
Total alcohols (fg/cell)	20.5	2.9	12.8	1.1	11.9	1.4	16.4	2.6
Long-chain diols								
$C_{30:0}$ 1,13 diol	1.7	0.8	1.0	0.7	0.7	0.4	3.1	1.9
$C_{30:0}$ 1,15 diol	1.8	0.6	1.2	0.4	1.5	0.3	1.9	0.7
$C_{31:0}$ 1,15 diol	1.2	0.7	1.3	0.5	1.4	0.5	1.7	1.4
$C_{32:1}$ 1,15 diol	8.8	2.7	6.7	1.6	2.5	1.4	10.5	2.5
$C_{32:0}$ 1,15 diol	18.4	6.9	10.3	4.7	22.6	9.0	26.3	10.3
$C_{34:0}$ 1,15 diol	tr	tr	tr	tr	tr	tr	tr	tr
Total diols (fg/cell)	31.9	11.7	20.5	7.9	28.7	11.6	43.5	16.8

*"Acid" indicates compounds identified after acid hydrolysis of the total extractable lipids; "Base" indicates compounds found after base hydrolysis.

Studies of the effects of different culture conditions

(i) *Fatty acids in Isochrysis* sp. (*clone T.iso)*

This alga was grown at different light intensities and harvested during log phase and stationary phase growth to determine how changes in environmental variables affect fatty acid compositions (Fig. 4; Table 4). The concentration of fatty acids per cell (Fig. 4), or as a percentage of total fatty acids (Table 4), was little affected by changes in light intensity, but at lower light intensities (50 μE.m^{-2}.s^{-1}) the concentration as a percentage of dry weight was twice as great. The relative abundance of 22:6(n–3) increased slightly as light intensity increased.

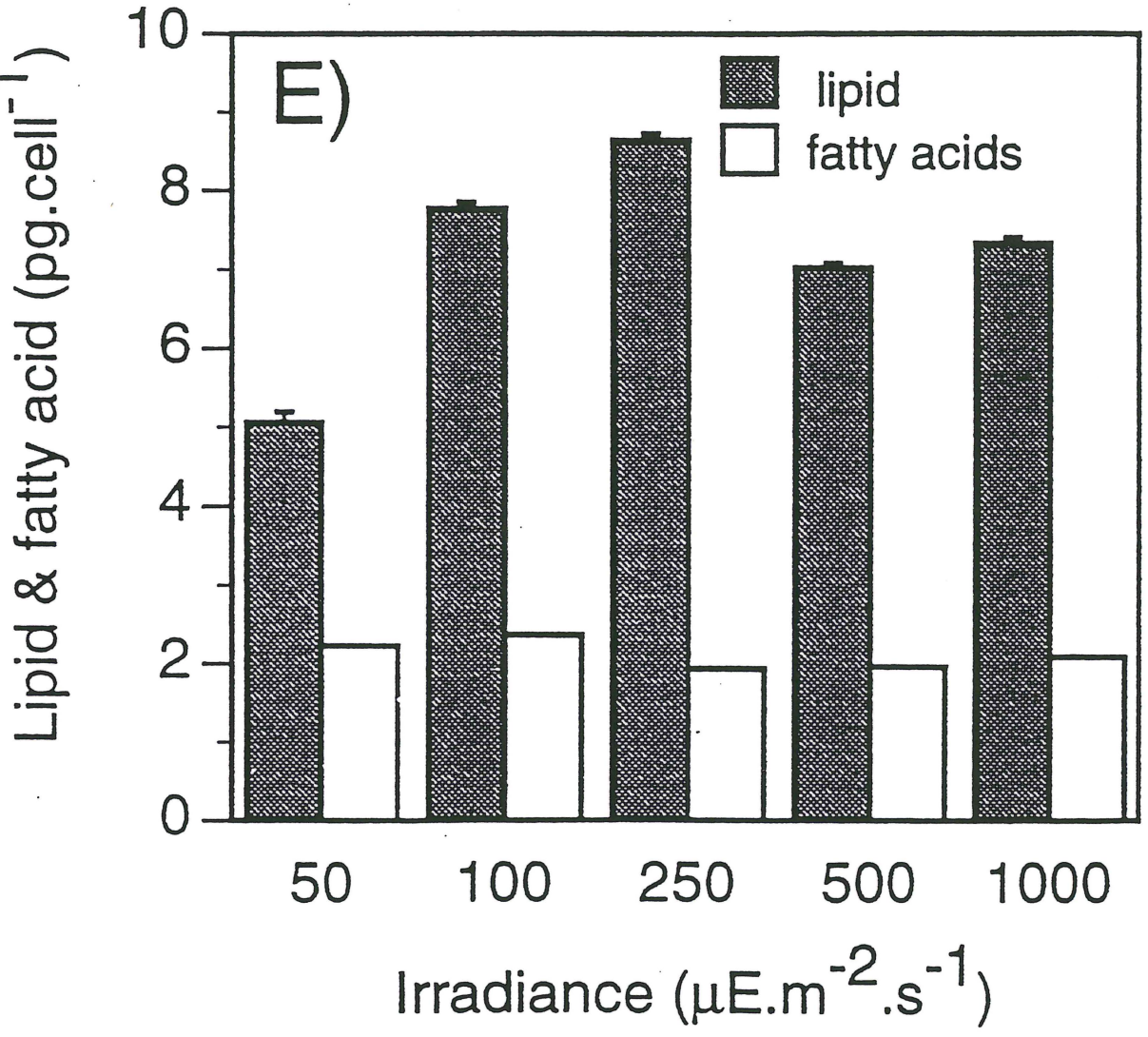

Figure 4. Concentrations of total lipid and total fatty acids in Isochrysis sp. (T.Iso) grown at different light intensities.

Table 4. Percentage compositions of major fatty acids in Isochrysis sp. (T.Iso) grown at different light intensities.

Lipid component	Irradiance ($\mu E \cdot m^{-2} \cdot s^{-1}$)					
	50	100	250 (A) [e]	250 (B) [e]	500	1000
Saturated fatty acids (percent of total fatty acids)						
14:0	17.8	17.0	17.0	17.4	17.3	16.5
15:0	0.8	0.4	0.9	0.8	1.2	0.8
16:0	10.5	11.4	13.3	13.3	12.1	12.8
18:0	0.2	0.3	0.5	0.3	0.2	0.2
Subtotal [a]	29.5	29.6	32.4	32.4	31.3	30.9
Monounsaturated fatty acids (percent of total fatty acids)						
16:1(n-9)	0.1	0.2	0.1	0.1	0.1	0.2
16:1(n-7)	5.0	4.9	5.8	5.6	5.5	5.1
17:1(n-8)	0.3	0.1	0.2	0.2	0.4	0.2
18:1(n-9)	7.3	8.5	11.4	11.6	10.6	10.7
18:1(n-7)	1.1	1.1	1.4	1.5	1.5	1.4
Subtotal [a]	13.9	14.9	19.2	19.2	18.2	17.7
Polyunsaturated fatty acids (percent of total fatty acids)						
16:2(n-7)	0.8	1.1	1.1	1.1	1.0	1.1
16:2(n-4)	0.3	0.2	0.3	0.3	0.2	0.2
16:3(n-4)	0.3	0.3	0.2	0.2	0.2	0.2
18:2(n-6)	2.5	1.9	1.6	1.7	1.5	1.8
18:3(n-6)	0.7	0.5	0.3	0.3	0.2	0.2
18:3(n-3)	6.2	5.5	4.8	5.0	4.2	4.2
18:4(n-3)	24.9	26.0	20.1	20.6	19.4	19.1
18:5(n-3)	5.9	5.6	4.7	4.8	5.7	5.9
20:5(n-3)	0.4	0.4	0.4	0.4	0.5	0.3
22:5(n-6)	1.7	1.5	1.5	1.5	1.7	1.9
22:6(n-3)	12.5	12.3	12.9	12.0	15.0	15.8
Subtotal [a]	56.6	55.5	48.4	48.4	50.2	51.2
Unidentified	tr [d]	tr	tr	tr	0.2	0.2
Total percent	100.0	100.0	100.0	100.0	100.0	100.0
Total concentration of fatty acids						
$pg.cell^{-1}$	2.22	2.36	1.88	1.95	1.95	2.07
% dry wt	7.37	5.25	3.32	3.44	3.15	3.87

(ii) *Effect of culture age*

Three algae (*Pavlova lutheri*, *Nannochloropsis oculata* and *Isochrysis* sp.) were grown in 100 litre bag culture using the joint University of Tasmania–CSIRO Division of Fisheries – Shellfish Culture Pty Ltd experimental research facility at Pipeclay Lagoon near Hobart. The cultures were harvested during exponential growth and stationary phase. Major changes in lipid composition occurred as the cultures aged (Fig. 5). In particular, the concentrations of triacylglycerols was greater, and the relative concentrations of PUFA was slightly less, in the stationary phase cultures. These findings indicate that the way algae are cultured at the hatchery can have a significant effect on the biochemical composition and hence nutritional quality of the microalgae.

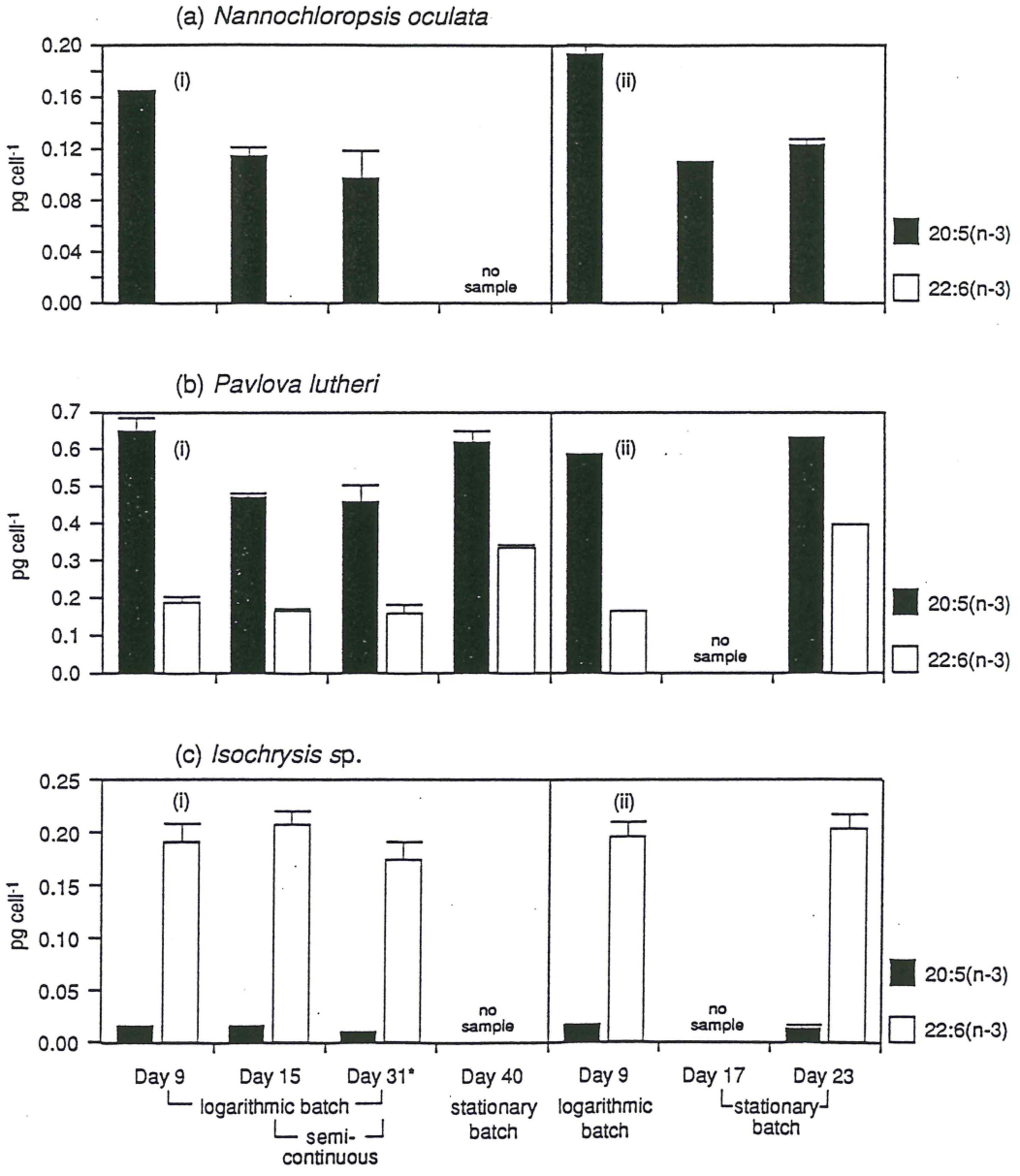

Figure 5. Concentrations of essential polyunsaturated fatty acids 20:5 and 22:6 in 3 microalgae harvested at different growth stages.

Mariculture feeding experiments

Our first trial experiments with oyster feeding were carried out at the research facility at Pipeclay Lagoon to assess feeding rations and digestibility of various algal species. The lipids and fatty acids of oyster broodstock were examined to provide a guide as to desirable levels to be attained under hatchery conditions. The efficiencies of different extraction methods were also examined, and it was found that freeze drying prior to extraction using standard methods led to significant losses of triacylglycerols and PUFA. A modified extraction method involving rehydration of the sample was developed which gave good yields of triacylglycerols and total lipids. It is important that these quantities can be measured accurately since the survival rate of larvae has been linked to their lipid content. Our studies indicate that it is preferable to extract fresh or frozen samples rather than freeze–dried samples for best analytical results.

Studies of lipid uptake by rotifers were undertaken with staff from the Tasmanian State Institute of Technology in Launceston (now University of Tasmania) to follow up earlier work carried out for Sea Hatcheries in Queensland in which rotifers were fed on the artificial diet Frippak. Rotifers fed *Pavlova lutheri* contained high concentrations of PUFA, whereas rotifers raised on yeast were low in fat and deficient in essential fatty acids (Fig. 6). High contents of PUFA were obtained after just 3 hours of feeding with *Pavlova*, and there was little additional benefit gained from extended feeding over several days. A paper describing these results was published (Nichols *et al.*, 1989), and another article appeared in *Austasia Aquaculture* (Daintith and Nichols, 1990).

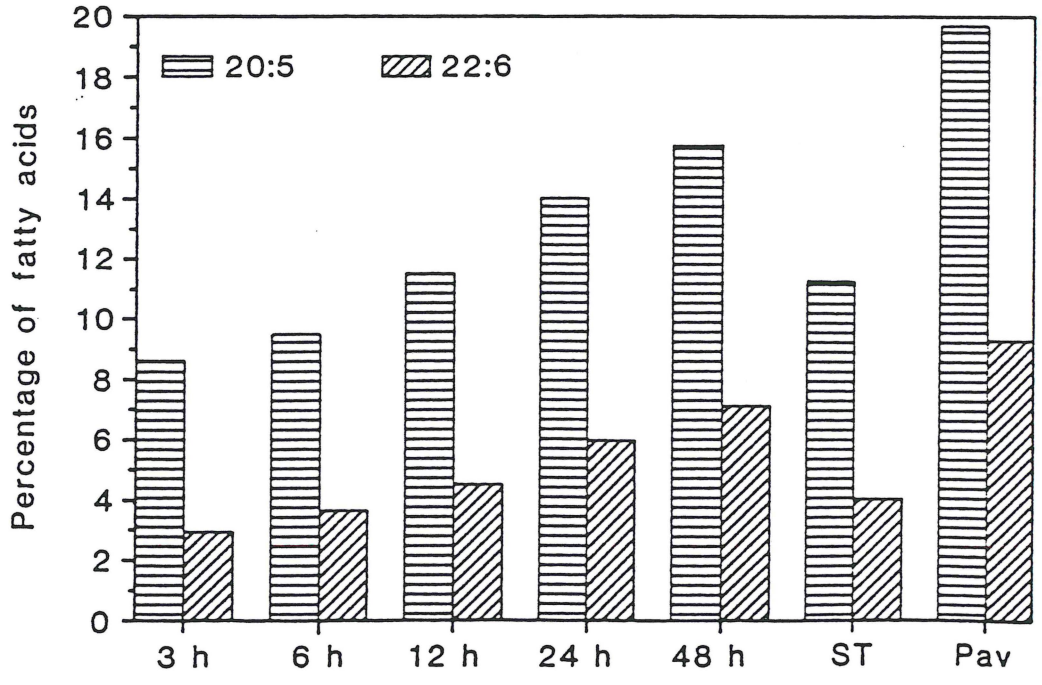

Figure 6. Percentage composition of 20:5 and 22:6 fatty acids in rotifers fed on the microalga *Pavlova lutheri* for 3–48 hours. Reproduced from Nichols *et al.* (1989).

Other research in support of the fishing industry

A number of projects proceeded in parallel with the mariculture experiments with the aim of providing data on the better utilisation of fish and crustacean by-catch as a source of commercially valuable chemicals and an alternative source of PUFA-rich materials for incorporation in mariculture feedstocks. These have included determination of the squalene content of deep-sea sharks caught off Tasmania (Deprez *et al.*, 1989), a baseline study of fatty acid contents in 21 commercial fish species commonly available in southern Australian markets (Bremner *et al.*, 1989), and analyses of the oil composition of orange roughy (Nichols *et al.*, 1989; Elliott *et al.*, 1990; Nichols and Elliott, 1990; Volkman and Skerratt, 1990). A separate proposal was funded by FIRDC to expand this work entitled "Orange roughy and other marine oils: characterisation and commercial applications" awarded to P. D. Nichols, J. K Volkman and N. Elliott.

Transfer of Results to Industry

A key component of our work has been the rapid transfer of our data to the mariculture industry. We have achieved this through collaborative projects with commercial companies (Tassal, Frish, Sea Hatcheries, Dalgety), other research organisations (CSIRO Division of Fisheries in Hobart, Perth and Cleveland, TSIT in Launceston, Marine Science Laboratories in Queenscliff) and Government Departments as well as through extensive personal contacts and visits to hatcheries.

Most of the fatty acid data obtained were distributed directly to the aquaculture industry as articles in the regular newsletter "Microalgae for Mariculture" compiled by Drs Jeffrey and Garland and funded by FIRTA Grant 86/81. This newsletter was regularly distributed to over 300 individuals and companies involved with mariculture, including major research and teaching organizations.

Our results were communicated to mariculturists attending the "Micro-algae for Mariculture" workshops held at the University of Tasmania on July 3-7, 1989 and February 19-23, 1990. Attendees were also given a guided tour of facilities at the CSIRO Marine Laboratories during which many informal discussions with industry personnel were held.

Staff from the project manned a trade exhibition showing the use of microalgae for mariculture at the Tasmanian Aquaculture Conference and Trade Show, Hobart July 14-16, 1989. Dr Jeffrey gave an overview lecture entitled "Microalgal feed in Australian mariculture".

Drs Volkman, Nichols, Jeffrey and Garland all presented papers at the *Aquaculture Nutrition Workshop*, Salamander Bay, New South Wales from April 15–17, 1991. Three papers were published in the Proceedings edited by Geoff Allan and Bill Dall.

Drs Volkman and Nichols presented data on algal lipids and feeding experiments at the *Australian Marine Sciences Conference* in Sydney, at the *Australian Organic Geochemistry Conference* in Hobart, at the *Fats for the Future II* Conference in Auckland, New Zealand, at the *Perspectives in Marine Natural Products* Conference in Auckland and at the *European Association for Organic Geochemistry* meeting in Paris.

A full list of publications arising out of the work (including some published in 1992/93 after the grant funding ended from work commenced during the grant) is shown in Appendix 2.

Appendix 1: List of the microalgal species from the CSIRO Algal Culture Collection that were examined for fatty acid content.

Species name	CSIRO code	Species name	CSIRO code
CHLOROPHYCEAE (green algae)			
Dunaliella tertiolecta	CS–175	*Nannochloris atomus*	CS–183
Stichococcus sp.	CS–92	*Chlorella* sp.	CS–195
Chlorella protothecoides	CS–41	*Chlorella* sp.	CS–247
PRASINOPHYCEAE (green algae)			
Tetraselmis suecica	CS–187	*Tetraselmis chui*	CS–26
Pyramimonas cordata	CS–140	*Micromonas pusilla*	CS–98
Micromonas sp.	CS–170	Unidentified sp.	CS–126
Pycnococcus provasolii	CS–185		
BACILLARIOPHYCEAE (diatoms)			
Chaetoceros calcitrans	CS–178	*Chaetoceros gracilis*	CS–176
Skeletonema costatum	CS–181	*Thalassiosira pseudonana*	CS–173
Nitzschia closterium	CS–5c	*Skeletonema* sp.	CS–252
Navicula sp.	CS–46c		
PRYMNESIOPHYCEAE (prymnesiophytes, golden brown flagellates)			
Isochrysis sp. (Tahitian)	CS–177	*Pavlova lutheri*	CS–182
Pavlova sp.	CS–50	*Pavlova salina*	CS–49
Pavlova sp.	CS–63		
CRYPTOPHYCEAE (cryptomonads)			
Chroomonas salina	CS–174		
EUSTIGMATOPHYCEAE (yellow–green algae)			
Nannochloropsis oculata	CS–216	*Nannochloropsis salina*	CS–190
Nannochloropsis sp.	CS–246	*Nannochloropsis oculata*	CS–179
RHODOPHYCEAE (red algae)			
Rhodosorus sp.	CS–210		

Appendix 2: PUBLICATIONS ON MARICULTURE NUTRITION FUNDED WHOLLY OR IN PART BY FIRTA GRANT 88/69

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

Dunstan, G. A., Volkman, J. K., Jeffrey, S. W. and Barrett, S. M. (1992) Biochemical composition of microalgae from the green algal classes Chlorophyceae and Prasinophyceae 2. Lipid classes and fatty acids. *J. Exp. Mar. Biol. Ecol.* **161**, 115–134.

Dunstan, G. A., Volkman, J. K. and Barrett, S. M. (1993) The effect of lyophilization on the lipid composition in adult oyster *Crassostrea gigas* (Thunberg). *Lipids* (in press)

Nichols, P. D., Holdsworth, D. G., Volkman, J. K., Daintith, M. and Allanson, S. (1989) High incorporation of essential fatty acids by the rotifer *Brachionus plicatilis* fed on the prymnesiophyte alga *Pavlova lutheri*. *Aust. J. Mar. Freshw. Res.* **40**, 645–655.

Volkman, J. K. (1989) Fatty acids of microalgae used as feedstocks in aquaculture. In *Fats for the Future* (ed. R. C. Cambie), pp. 263–283.

Volkman, J. K., Jeffrey, S. W., Nichols, P. D., Rogers, G. I. and Garland, C. D. (1989) Fatty acid and lipid composition of 10 species of microalgae used in mariculture. *J. Exp. Mar. Biol. Ecol.* **128**, 219–240.

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

Volkman J. K., Dunstan G. A., Jeffrey S. W. and Kearney P. S. (1991) Fatty acids from microalgae of the genus *Pavlova*. *Phytochemistry* **30**, 1855–1859.

Volkman, J. K., Dunstan, G. A., Barrett, S. M., Nichols, P. D. and Jeffrey, S. W. (1992) Essential polyunsaturated fatty acids of microalgae used as feedstocks in aquaculture. In *Proceedings Aquaculture Nutrition Workshop*, Salamander Bay, New South Wales. (Edited by G. L. Allan and W. Dall). pp. 180–186.

Volkman, J. K., Brown, M. R., Dunstan, G. A. and Jeffrey, S. W. (1993) Biochemical composition of marine microalgae from the class Eustigmatophyceae used in mariculture. *J. Phycol.* **29**, 69–78.

Magazine and newsletter articles relating to this research

Summary of the fatty acid composition of ten species of micro-algae held in the CSIRO algal culture collection, and used by the Australian mariculture industry. *Micro-algae for Mariculture Newsletter* No. 3(1-2), 1987.

Nutritional studies- polyunsaturated fatty acids in microalgae. *Micro-algae for Mariculture Newsletter* No. 5(6). 1989.

Q: When is a "*Chlorella*" not a *Chlorella*? A: When it is *Nannochloropsis oculata*. *Micro-algae for Mariculture Newsletter* No. 7(3). 1990.

New *Pavlova* species for mariculture. *Micro-algae for Mariculture Newsletter* No. 7(4). 1990.

The effects of light on nutritional quality of *Isochrysis* sp. (clone T.Iso; CS-177). *Micro-algae for Mariculture Newsletter* No. 7(5). 1990.

Enrichment of rotifers with polyunsaturated fatty acids (PUFA). *Micro-algae for Mariculture Newsletter* No. 7(6). 1990.

Nutritional aspects of the rotifer *Brachionus plicatilis*. *Austasia Aquaculture* **4**: 16-17. 1990.

Bivalve research facility to overcome industry problems. *Austasia Aquaculture* **5**: 10-11. 1991.

Microalgae; nutritious live food. *Austasia Aquaculture* **5**, 18-19. 1991.

PUBLICATIONS ON MARINE LIPIDS (CARRIED OUT IN CONJUNCTION WITH FIRTA 88/69)

Bremner, H. A., Volkman, J.K., Krasnicki, T. and Gibson, R. (1989) The good oil: nutritionally important oils in Tasmanian fish. *Aust. Fisheries* **48(5)**, 28–29.

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

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

Elliott, N., Skerratt, J. and Nichols, P. D. (1990) Orange roughy oil proves its worth. *Aust. Fisheries* **49(8)**, 32–33.

Hallegraeff, G. M., Nichols, P. D., Volkman, J. K., Blackburn, S. I. and Everitt, D. A. (1991) Pigments, fatty acids, and sterols of the toxic dinoflagellate *Gymnodinium catenatum*. *J. Phycol.* **27**, 591–599.

Nichols, P., Skerratt, J., Elliott, N. and Volkman, J. (1989) A simple procedure to fully identify monounsaturated alkyl chains in marine wax esters. In *Food Forums Proceedings,; Tenth Australian Symposium on Analytical Chemistry, Brisbane, August 1989* pp. 185–190.

Volkman, J. K., Kearney, P. and Jeffrey, S. W. (1990) A new source of 4–methyl sterols and 5α(H)–stanols in sediments: prymnesiophyte microalgae of the genus *Pavlova*. *Org. Geochem.* **15**, 489–497.

Volkman, J. K., Barrett, S. M., Dunstan, G. A. and Jeffrey, S.W. (1992) C_{30}–C_{32} alkyl diols and unsaturated alcohols in microalgae of the class Eustigmatophyceae. *Org. Geochem.* **18**, 131–138.

INDUSTRY REPORTS BY VOLKMAN AND NICHOLS ON MARINE LIPIDS AND MARICULTURE 1988–91.

Nichols, P. D. (1989) Lipid and fatty acid composition of prawn feeds. Report 89–CSCL1 for Dalgety Pty. Ltd..

Nichols, P. D. and Elliott, N. (1990) Lipid, fatty acid and fatty alcohol composition of WINI Orange roughy oil. Report 90–WIN1 to WINI Pty. Ltd., Victoria.

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

Nichols, P. D. (1991) Lipid, fatty acid and fatty alcohol composition of Trident Dory oil. Report 91–Trident1 to Trident Seafoods, Tasmania.

Nichols, P. D. (1991) The composition of new Trident oils produced from: I. whole shark and II. Salmon farm waste. Report 91–Trident3 to Trident Seafoods, Tasmania.

Volkman, J. K. and Holdsworth, D. G. (1988) Fatty acid composition of algal meal. Report 88–FA3 for New South Wales Department of Agriculture.

Volkman, J. K., Holdsworth, D. and Dunstan G. (1989a) Fatty acid composition of rotifers and *Artemia* fed on Frippak supplements. Report 89–FA3 for Sea Hatcheries Ltd., Queensland.

Volkman, J. K. and Barrett, S. M. (1990) Lipid composition of fish, worms and mussels from Sydney waters. Report 90–JET1/R for Johnstone Environmental Technology Pty Ltd, Sydney.

Volkman, J. K. and Skerratt, J. (1990) Wax ester, fatty acid and alcohol composition of Trident orange roughy oil. Report 90–ORT1 for Trident Seafoods, Hobart.

Volkman, J. K. and Barrett, S. M. (1991) Lipid composition of crabs, prawns and fish from Sydney waters. Report 91–JET1 for Johnstone Environmental Technology Pty Ltd, Sydney.

Volkman, J. K., O'Leary, T. and Barrett, S. (1991) Fatty acid composition of fifteen samples of *Artemia* fed on various microalgal diets. Report 91–MBH for Mahanga Bay Hatchery, Wellington, New Zealand.

Conference and Workshop Presentations (1988–91)

In chronological order:

P. D. Nichols and J. K. Volkman *Lipids of microalgae: taxonomic and environmental applications.* Marine Phytoplankton Symposium, Hobart, 1988.

P. D. Nichols and J. K. Volkman *Lipid assays in mariculture and environmental studies.* Australian Marine Sciences Silver Jubilee Conference, Sydney, December 14–16, 1988.

P. D. Nichols and J. K. Volkman. *Rotifer feeding experiments: incorporation of essential fatty acids by Brachionus plicatilis fed on Pavlova lutheri.* Fats for the Future II Conference, Auckland, New Zealand, February 12–17, 1989.

J. K. Volkman. *Fatty acids of microalgae used in aquaculture.* Fats for the Future II Conference, Auckland, New Zealand, February 12–17, 1989.

P. D. Nichols, D. Holdsworth, M. S. Rayner and J. K. Volkman (1989) *The use of lipid assays in mariculture and environmental studies.* Australian Organic Geochemistry Conference, Hobart, April 13–14, 1989.

J. K. Volkman. *Lipids in mariculture feedstocks and their role in human nutrition.* Micro–algae for Mariculture Workshop, University of Tasmania, July 3–7, 1989.

S. W. Jeffrey. *Microalgal feed in Australian mariculture.* Tasmanian Aquaculture Conference and Trade Show, Hobart July 14–16, 1989.

P. Nichols, J. Skerratt, N. Elliott and J. Volkman (1989). *A simple procedure to fully identify monounsaturated alkyl chains in marine wax esters*. Chemistry International Conference, Food Forums Proceedings, Brisbane, August 29 and 31, 1989.

J. K. Volkman, P. D. Nichols, P. S. Kearney, G. I. Rogers and S. W. Jeffrey. *4–Methyl sterols and 5α(H)–stanols in some prymnesiophyte microalgae.* 14th International Meeting on Organic Geochemistry, Paris, France, September 18–22, 1989.

P. D. Nichols *Lipids in mariculture feedstocks and their role in human nutrition.* Micro–algae for Mariculture Workshop, University of Tasmania, Feb. 20, 1990.

J. K. Volkman, P. D. Nichols, G. A. Dunstan, S. M. Barrett and S. W. Jeffrey (1991) *Novel aspects of the lipid biochemistry of marine microalgae: applications to geochemistry, mariculture and taxonomy.* Perspectives in Marine Natural Products Conference, Auckland, February 7–8, 1991.

J. K. Volkman and S. M. Barrett (1991) *An algal source for C_{30}–C_{32} alkyl diols in the marine environment.* Australian Organic Geochemistry Conference, Melbourne, April 11–12, 1991.

J. K. Volkman, G. A. Dunstan, S. M. Barrett, P. D. Nichols and S. W. Jeffrey (1991) *Essential polyunsaturated fatty acids of microalgae used as feedstocks in aquaculture.* Aquaculture Nutrition Workshop, Salamander Bay, New South Wales, April 15–17, 1991.

G. A. Dunstan, J. K. Volkman, S. M. Barrett and P. D. Nichols (1991) *Polyunsaturated fatty acids of microalgae grown in mass culture for mariculture.* Australian Marine Sciences Conference, Brisbane, Australia. July 8–11.

S. W. Jeffrey, M. R. Brown and J. K. Volkman (1991) *Microalgae for mariculture.* Fourth International Phycological Congress, Duke University, North Carolina USA, August 4–10.

J. K. Volkman *Lipids in mariculture feedstocks and their role in human nutrition.* Microalgae for Mariculture Workshop, University of Tasmania, December 2–6, 1991.