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Nutritional Aspects of Microalgae used in Mariculture; a Literature Review

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M. R. Brown*, S. W. Jeffrey* and C. D. Garland†

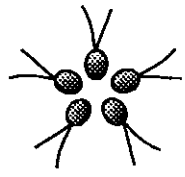
* CSIRO Division of Fisheries
Marine Laboratories
GPO Box 1538
Hobart, Tasmania 7001
Australia

† Department of Agricultural Science
University of Tasmania
GPO Box 252C
Hobart, Tasmania, 7001
Australia

Abstract

A literature review on the nutritional aspects of microalgae in the mariculture of bivalve molluscs, crustaceans and fish is presented. Differences are noted in the gross chemical composition of microalgae (total protein, carbohydrate, lipid and mineral) and levels of specific nutrients (amino acids, sugars, fatty acids, phospholipids, sterols, hydrocarbons, pigments, minerals and vitamins). An account is given of the current understanding of the nutritional requirements of animals. These nutritional requirements, together with the differences in biochemical composition of microalgae, explain why microalgae have supported animal growth to varying degrees. Documentation is provided for a range of algal diets that have been successful in promoting animal growth.

Other aspects, such as water conditioning by algae, bacteria, silt and artificial diets, which may contribute to animal nutrition in mariculture, are also discussed.



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Contents

1 Introduction

The culture of microalgae as food for the commercial rearing of marine animals is of critical importance in the mariculture industry. Microalgae (also termed "algae" in this review) are directly utilised by all growth stages of bivalves, by larval stages of some crustacean species, and by very early growth stages of some fish species. Algae are also used to rear mass quantities of zooplankton (rotifers, copepods and brine shrimp) for food for late-larval and juvenile stages of crustaceans and fish. The value of algae in this zooplankton food chain is also critical, since essential algal nutrients are passed on via the intermediary zooplankton to the cultivated animals. The central role of algae in the nutrition of maricultured animals is shown in Fig. 1.

Although many algal species have been used as food in mariculture operations, they are not equally successful in supporting the growth of a particular animal [43, 56, 138, 212, 213; see also Table 1]. The reasons for this are related to differences in the size, digestibility and particularly the nutritional value of the algae. The nutritional value depends primarily on the biochemical composition of the algae and the specific nutritional requirements of the feeding animal.

Because of the importance of knowing which are the most suitable algae to use as food for particular animals, we have reviewed the literature (to December 1988), and attempted to establish guidelines for the future. In the first section of this review, the gross chemical composition and level of specific nutrients in cultured algae are examined. The second section gives an evaluation of the nutritional needs of the animals (with reference to nutrients that can be provided by algae). Finally, factors important in the mass culture of algae, including the manipulation of the biochemical composition of algae by environmental factors and the potential of artificial diets to supplement or substitute for living algae, are presented.

The production of toxins and animal growth-inhibitors is beyond the scope of this review; the reader is referred to other publications where these aspects are discussed in detail [17, 83, 224].

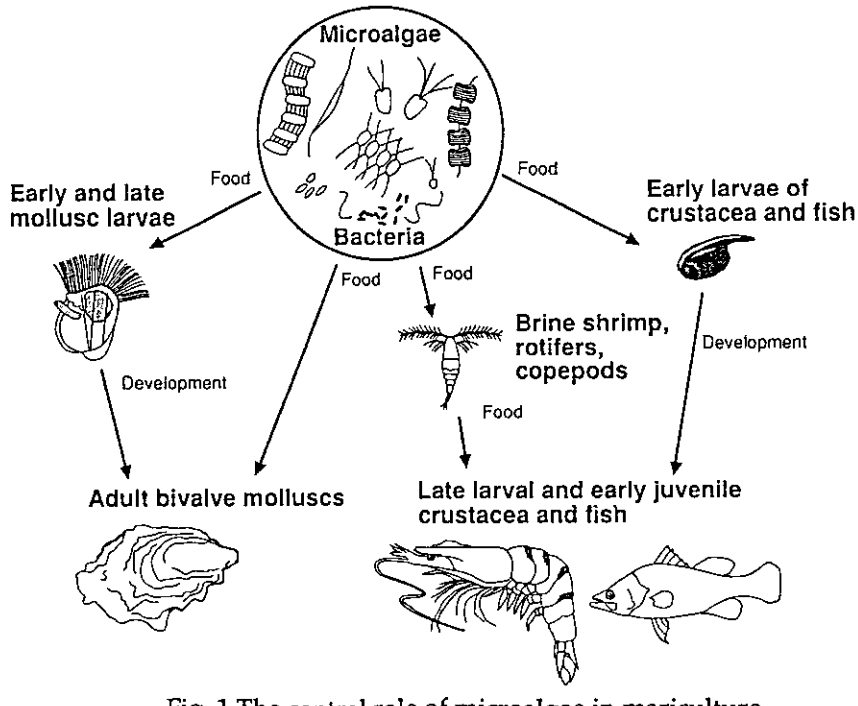

Fig. 1 The central role of microalgae in mariculture.

Table 1: Summary of commonly used microalgae in mariculture, their biochemical composition, and results of growth experiments with bivalve molluscs.

Codes used:

PUFA = polyunsaturated fatty acid; f.acid = fatty acid; a acid = amino acid; CHO = carbohydrate
For PUFA analysis: + = present as major component (>0.5% of total fatty acids); TR = trace component (<0.5% but detectable);
— = not detected;
? = information not available
For Growth Experiments: — = no literature found for that category

Note: Authority is not specified in the table if not given in the original reference.

BIOCHEMICAL COMPOSITION DATA ON ALGAE						GROWTH EXPERIMENTS USING SINGLE SPECIES OF ALGAE AS FOOD (SPECIES OF ANIMAL FED; [REFERENCE])	
	PUFA Analysis						
Algae/Class	22:6ω3	20:5ω3	Other PUFAs	Amino acid deficiencies	Analyses done [reference]	Good growth and survival	Poor growth and survival
Golden brown flagellates (PRYMNESIOPHYCEAE & CHRYSOPHYCEAE)							
Isochrysis galbana Parke	+	+	high 18:2ω6 high 18:3ω3 high 18:4ω3	no	f.acid [15, 209] CHO [29, 221] sterol [207] a.acid [26]	*Mytilus edulis* larvae [10] *Crassostrea virginica* juv. [43, 173] *Crassostrea gigas* juv. [213] and larvae [209] *Ostrea edulis* larvae [211, 225] *Ostrea edulis* juv. [213] *Ostrea lutaria* juv. [213] *Mercenaria mercenaria* larvae [43] *Mercenaria mercenaria* juv. [213]	—
Isochrysis sp. clone T-ISO	+	TR	high 18:4ω3	no	f.acid [167, 206] a.acid [56] CHO [221]	*Strombus gigas* larvae [167] *Crassostrea virginica* larvae [223] *Patinopecten yessoensis* larvae [139] *Ostrea edulis* juv. [56, 126] & larvae [223] *Mercenaria mercenaria* larvae [89, 223] *Tapes semidecussata* larvae [89]	*Crassostrea gigas* larvae [89] *Crassostrea rhizophorae* larvae [89]
Pavlova lutheri (Droop) Green	+	+	high 18:4ω3	no	f.acid [131, 206] CHO [29] sterol [9] a.acid [35]	*Mytilus edulis* larvae [10] *Ostrea edulis* larvae [211, 225] *Ostrea edulis* juv. [213] *Crassostrea virginica* juv. [43] *Crassostrea gigas* juv. [131, 213] *Mercenaria mercenaria* larvae [43]	*Ostrea edulis* juv. [56] *Ostrea lutaria* juv. [213] *Mercenaria mercenaria* juv. [213]
Pavlova gyrans Butcher	?	?	?	?	?	—	*Crassostrea gigas* juv. [213] *Ostrea edulis* juv. [213] *Mercenaria mercenaria* juv. [213]
Dictrateria inornata Parke	?	?	?	?	?	*Ostrea edulis* juv. [213] *Mercenaria mercenaria* juv. [213]	—
Crisophaera carterae (Braarud & Fagerland) Braarud	?	?	?	?	?	*Ostrea edulis* juv. [213]	—
Emiliania huxleyi (Lohm.) Hay & Mohler	+	TR	high 18:3ω3 high 18:4ω3 high 18:5ω3	?	f.acid [167, 207] sterol [207]	*Strombus gigas* larvae [167]	—
Pseudoisochrysis paradoxa Isol. Ott Va-12	+	TR	low	no	f.acid [27] CHO [29] sterol [137] a.acid [219]	—	*Ostrea edulis* larvae [225] *Ostrea edulis* juv. [56]
Olisthodiscus sp	?	?	?	?	?	—	*Mercenaria mercenaria* juv. [213] *Ostrea edulis* juv. [213]
Diatoms (BACILLARIOPHYCEAE)							
Chaetoceros calcitrans (Paulsen) Takano	+	+	high 20:4ω6	no	f.acid [206, 209] a.acid [56] CHO [221]	*Ostrea edulis* juv. [56, 126, 213] & larvae [225] *Crassostrea gigas* larvae [89, 209] *Crassostrea virginica* juv. & larvae [201] *Crassostrea rhizophorae* larvae [89] *Mercenaria mercenaria* larvae [89] *Tapes semidecussata* larvae [89]	—
Chaetoceros gracilis Schutt	TR	+	high 20:4ω6	no	f.acid [15, 206] a.acid [56]	*Ostrea edulis* juv. [56]	—
Chaetoceros simplex Ostenfeld (Bbam)	?	?	?	no	a.acid [32]	*Ostrea edulis* juv. [56]	—
Skeletonema costatum (Greville) Cleve	+	+	+	low Trp	f.acid [206] a.acid [32, 56] sterol [9] CHO [164]	*Ostrea edulis* juv. [56, 213] *Mercenaria mercenaria* juv. [213]	*Ostrea edulis* juv. [126]
Skeletonema menzelii Guillard, Carpenter & Reimann (Men)	?	?	?	no	a.acid [56]	*Ostrea edulis* juv. [56]	—
Phaeodactylum tricornutum (Bohlin)	+	TR	+	low Trp	f.acid [15, 61] a.acid [61] CHO [61] Sterol [161]	*Mytilus edulis* [123] *Ostrea edulis* larvae [225]	*Ostrea edulis* juv. [56, 213] *Crassostrea virginica* larvae & juv. [61]
Thalassiosira pseudonana - 3H (Hustedt) Hasle et	+	+	+	no	f.acid [205, 206] a.acid [61] sterol [61]	*Argopecten irradians* juv. [59] *Crassostrea virginica* juvenile [173] *Ostrea edulis* larvae [225]	

BIOCHEMICAL COMPOSITION DATA ON ALGAE						GROWTH EXPERIMENTS USING SINGLE SPECIES OF ALGAE AS FOOD (SPECIES OF ANIMAL FED; [REFERENCE])	
	PUFA Analysis						
Algae/Class	22:6ω3	20:5ω3	Other PUFAs	Amino acid deficiencies	Analyses done [reference]	Good growth and survival	Poor growth and survival
Heimdal						*Mytilus edulis* [59] *Tapes japonica* [76] *Mercenaria mercenaria* [58]	—
Thalassiosira fluviatilis Hustedt	?	?	?	?	?	—	*Mytilus edulis* juv. [213]
Green flagellates (PRASINOPHYCEAE & CHLOROPHYCEAE)							
Tetraselmis chuii Butcher	?	?	?	no	a.acid [58]	*Ostrea edulis* juv. [213]	—
Tetraselmis suecica (Kylin) Butcher	TR	+	high 16:4ω3 high 18:2ω6 high 18:3ω3 high 18:4ω3	no	f.acid [131,206] CHO [221] a.acid [58]	*Crassostrea gigas* larvae & juv. [131,201, 213] *Ostrea edulis* juv. [213] & larvae [225] *Mytilus edulis* juv. [213]	*Crassostrea virginica* juv. [173] *Ostrea edulis* juv. [216] *Mercenaria mercenaria* juv. [213]
Tetraselmis maculata Butcher	?	?	?	no	a.acid [164] CHO [164]	*Ostrea edulis* juv. [56] *Crassostrea virginica* juv. [223]	—
Tetraselmis tetrahele (G.S. West) Butcher	?	?	?	?	?	*Ostrea edulis* juv. [213]	—
Tetraselmis inconspicua Butcher	?	?	?	?	?	*Ostrea edulis* juv. [213]	—
Micromonas pusilla Butcher	?	?	?	?	?	—	*Mercenaria mercenaria* juv. [213] *Ostrea edulis* juv. [213]
Dunaliella tertiolecta Butcher	—	—	high 16:4ω3 high 18:3ω3	low Trp	f.acid [131, 167] a.acid [56, 219]	—	*Ostrea edulis* juv. [56, 213] *Crassostrea gigas* juv. [131] *Crassostrea virginica* juv. [223] *Strombus gigas* larvae [167] *Mercenaria mercenaria* juv. [213]
Dunaliella euchlora Lerche	?	?	?	?	?	*Crassostrea virginica* larvae [43]	*Ostrea edulis* juv. [213] *Mercenaria mercenaria* larvae [43] *Mercenaria mercenaria* juv. [213]
Nannochloris atomus Butcher	TR	+	high 16:3ω3 high 18:2ω6 high 18:3ω3	?	f.acid [206]	—	*Ostrea edulis* juv. [213] *Mytilus edulis* larvae [10]
Chlamydomonas coccoides Butcher	?	?	?	?	?	—	*Mercenaria mercenaria* juv. [213] *Ostrea edulis* juv. [213]
Branchiomonas sub-marina Bohlin	?	?	?	no	a.acid [35]	—	*Mercenaria mercenaria* juv. [213] *Ostrea edulis* juv. [213]
Pyramimonas virginica Pennick	+	+	high 18:3ω3 high 18:4ω3	low Met	f.acid [27] CHO [29] a.acid [219]	*Crassostrea gigas* larvae [209]	—
Chlorella autotrophica var. *atypica* Shihara & Krauss	?	?	?	?	?	—	*Argopecten irradians concentricus* [165] *Ostrea edulis* juv. [213]
Chlorella stigmatophora Butcher	—	+	+	?	f.acid [15]	—	*Ostrea edulis* larvae [211] *Ostrea edulis* juv. [213] *Crassostrea gigas* juv. [213] *Mercenaria mercenaria* juv. [213] *Mytilus edulis* juv. [213]
Cryptomonads (CRYPTOPHYCEAE)							
Rhodomonas sp. Rhodo	?	?	?	?	?	*Ostrea edulis* juv. [56]	—
Chroomonas salina (Wislough) Butcher	+	+	high 18:3ω3 high 18:2ω6 high 18:4ω3	?	f.acid [206]	*Ostrea edulis* spat [126]	—
Cryptomonas sp.	?	?	?	?	?	—	*Ostrea edulis* juv. [213]
Dinoflagellates (DINOPHYTA)							
Prorocentrum minimum (Pavillard) Schiller clone EXUV	+	+	high 18:5ω3	?	f.acid [167]	*Strombus gigas* larvae [167]	—
Heterocapsa pygmaea (Loeblich) Schmidt & Sherley, clone Gymno	+	+	high 18:4ω3	?	f.acid [167]	*Strombus gigas* larvae [167]	—
Symbiodinium microadriaticum	?	?	?	?	?	*Tridacna gigas* larvae & juv. [71] *Hippopus hippopus* larvae & juv. [71]	—

2 Biochemical Composition of Algae

Gross Composition

Protein, carbohydrate, lipids and minerals make up 90–95% of the dry weight of an algal cell. The remainder is accounted for by nucleic acids (5–10%) [13, 65, 67]. Typical values for the gross chemical composition of a number of algae are shown in Table 2.

Variables such as photoperiod, light intensity, colour (wavelength), temperature, type of nutrients in culture media and stage of growth at harvest can influence gross composition [64, 66, 78, 79, 92, 149, 176, 215, 221]. This aspect is discussed in detail in chapter 4.

Table 2: Gross chemical composition (% dry weight) of microalgae commonly used in mariculture. All values are from algae harvested during the exponential growth phase.

Class and Algal species	Common name	Protein* (%)	CHO (%)	Lipid (%)	Mineral (%)	Total† (%)	Reference
Prymnesiophyceae							
Isochrysis sp. clone T-ISO	golden-brown flagellate	44	9	25	9	87	[221]
Isochrysis galbana	golden-brown flagellate	41	5	21	13	80	[221]
Pavolva lutheri	golden-brown flagellate	49	31	12	6	98	[164]
Bacillariophyceae							
Chaetoceros calcitrans	diatom	33	17	10	29	89	[201]
Phaeodactylum tricornutum	diatom	33	24	10	8	75	[164]
Skeletonoma costatum	diatom	37	21	7	39	104	[164]
Thalassiosira pseudonana	diatom	29	17	10	38	94	[221]
Chlorophyceae							
Dunaliella salina	green flagellate	57	32	9	8	106	[164]
Prasinophyceae							
Tetraselmis suecica	green flagellate	39	8	7	23	77	[221]

*"Crude protein"; determined as N x 6.25

†The deviation from 100% for the total sum of the different metabolites is attributed to inaccuracies of analysis and estimation, as outlined by Parsons *et al.* [164]

Essential Components

The quality of gross biochemical fractions is determined by the proportion and availability of specific components making up those fractions, as detailed below.

Amino Acids

The nutritional value of protein is determined by the content and availability of its constituent amino acids. Of the total amino acids in algae, 90–98% occur in protein [53]. A number of investigators have analysed the total amino acid composition of algae, generally from whole cell hydrolysates [26, 32, 35, 56, 61, 87, 164].

The proportions of individual amino acids do not vary greatly between different algal species (Table 3). Differences in the nutritional quality of algae are therefore, in most instances, unrelated to amino-acid composition [219]. (For further details see chapter 3).

The amino-acid composition of algae is quite similar to that of chicken egg protein (which is considered of high biological value in human nutrition), although the latter is richer in methionine and lower in arginine [197].

Table 3: Amino-acid composition (g/100 g of total amino acid fraction in hydrolysate) of some microalgae commonly used in mariculture. Hydrolysates were prepared from whole algae, except those indicated by *, which were prepared from extracted protein.
All values are from algae harvested during the exponential growth phase, except those indicated by *, which were taken from algae harvested during the stationary phase.
n.d. = not determined; + = present (but not quantified)

	Algal species								
Amino acid	*Isochrysis galbana*	*Isochrysis* sp. clone T-ISO	*Pavlova lutheri*	*Chaetoceros calcitrans*	*Phaeodactylum tricornutum**	*Skeletonema costatum*	*Thalassiosira pseudonana**	*Dunaliella salina*	*Tetraselmis suecica**
threonine	5.0	5.8	3.6	5.9	5.7	5.5	4.2	6.3	3.6
valine	6.8	6.5	5.0	5.9	6.4	5.5	6.7	4.2	7.1
methionine	3.2	2.1	1.6	2.3	1.9	1.3	2.3	+	2.0
isoleucine	3.3	4.8	2.9	5.5	5.1	5.9	5.6	+	4.8
phenylalanine	4.4	5.4	2.8	5.9	5.7	5.5	6.0	4.5	5.9
lysine	7.3	6.7	8.2	7.3	5.1	7.9	6.3	12.0	6.6
histidine	1.9	2.3	3.6	2.3	1.4	2.1	2.4	n.d.	2.2
arginine	5.7	7.8	11.3	6.4	9.6	6.1	6.6	n.d.	6.4
proline	6.7	n.d.	3.2	n.d.	4.4	n.d.	4.9	+	3.7
leucine	10.2	9.7	6.7	9.1	8.9	8.4	9.6	+	3.2
tyrosine	2.1	4.0	2.2	4.5	3.2	3.9	3.6	+	9.7
tryptophan	0.4	n.d.	1.7	n.d.	0.0	n.d.	0.4	n.d.	0.5
alanine	9.7	8.1	8.3	7.7	8.3	9.2	6.8	19.0	8.7
aspartate	9.9	11.1	6.1	11.4	10.8	11.3	10.3	16.5	10.0
cystine	0.5	n.d.	1.1	n.d.	0.6	n.d.	1.0	n.d.	0.4
glutamate	8.4	15.1	6.3	15.0	13.4	16.9	11.2	13.6	13.5
glycine	6.3	5.3	6.7	5.5	7.0	5.5	7.0	16.8	7.4
serine	6.0	5.4	4.3	5.9	5.1	5.5	5.0	n.d.	4.3
Reference:	[26]	[56]	[35]	[56]	[61]	[56]	[61]	[164]	[58]

Some amino acids may be unavailable for animal digestion and absorption if sections of the molecule are bound to other molecules. For example, the free-amino group of lysine can sometimes be bound to carbohydrate. This reaction is common during processing of harvested algae (e.g. drying), and should be taken into consideration if dried algae are used as animal food. Most chemical methods of amino-acid analysis do not differentiate available from unavailable (blocked amino) lysine, although one report has quoted an 85% availability for lysine in dried *Spirulina* sp. [171].

Carbohydrate

Few detailed analyses have been made of the carbohydrate composition of algal species [29, 164, 221]. The total carbohydrate fraction is composed of the polysaccharide fraction (which may constitute from 45–97% of the total carbohydrate fraction [221]) and mono-saccharides and oligo-saccharides. Carbohydrate profiles of algal species vary widely (Table 4). The principal sugars are glucose, galactose, mannose and ribose, with other sugars in varying proportions.

Differences have also been demonstrated in the nature of the polysaccharides found in algae. For example, diatom polysaccharides predominantly contain chrysolaminarin (a ß1–3 glucan) and mannans [149, 221], whereas phytoflagellates contain glucans, principally of glucose and galactose [221]. Red algae accumulate high levels of sulphated polysaccharides [54].

Table 4: Carbohydrate composition (g/100 g of total monosaccharide in hydrolysate fraction) of some microalgae commonly used in mariculture.
Hydrolysates were prepared from whole algae, except those indicated by *, which were prepared from polysaccharide component.
All values are from algae harvested during exponential growth phase.
– = not detected

	Algal species								
Sugar Component	*Isochrysis galbana*	*Isochrysis* sp. clone T-ISO	*Pavlova** *lutheri*	*Chaetoceros calcitrans*	*Phaeodactylum tricornutum*	*Skeletonema costatum*	*Thalassiosira pseudonana*	*Dunaliella salina*	*Tetraselmis suecica*
rhamnose	1.0	0.2	1.4	1.1	6.7	5.1	0.6	—	0.3
fucose	0.8	0.6	3.8	5.3	2.3	5.8	—	—	0.3
ribose	48.2	13.2	5.3	10.9	2.8	0.6	3.1	0.5	3.8
xylose	6.9	3.9	—	1.1	10.9	4.3	0.8	—	0.2
arabinose	3.4	9.8	—	—	3.1	3.3	—	—	0.7
mannose	11.8	22.3	15.2	1.4	33.2	30.3	4.0	—	19.0
galactose	13.7	18.7	—	12.4	5.5	2.0	5.3	37.3	32.2
glucose	12.7	31.3	67.2	66.3	35.1	48.6	86.2	54.4	43.5
inositol	1.5	0.1	—	1.6	—	—	—	—	—
glycerol	—	—	1.8	—	—	—	—	—	—
fructose	—	—	0.6	—	—	—	—	—	—
ribitol/xylitol	—	—	2.1	—	—	—	—	—	—
Reference:	[221]	[221]	[29]	[221]	[84]	[84]	[221]	[164]	[221]

Lipids

The lipid fraction can be divided into two categories: polar lipids (which include the phospholipid and glycolipid fractions) and neutral lipids (which include the triglycerides, diglycerides, hydrocarbons, alkenones, sterols and pigments). The structures of the most important of these lipid classes are shown in Figures 2 and 3.

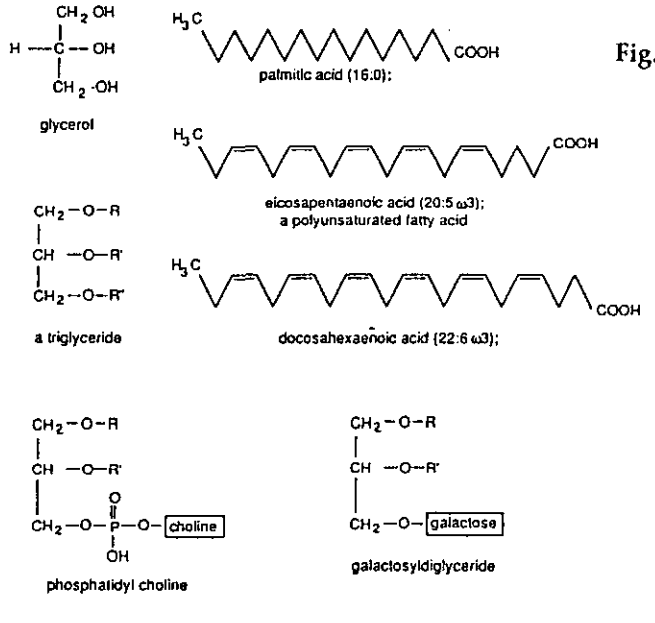

Fig. 2: Structures of lipid components of algae.triglycerides are esters of glycerol and fatty acids (e.g. 16:0,20:5ω3 and 22:6ω3). Phosphatidylcholine (a phospholipid) and galactosyldiglyceride (a glycolipid) have a similar structure to triglyceride, except that the fatty acid in the carbon-3 position is replaced by a phosphocholine and galactose, respectively.
R, R' and R'' represent fatty acid groups.

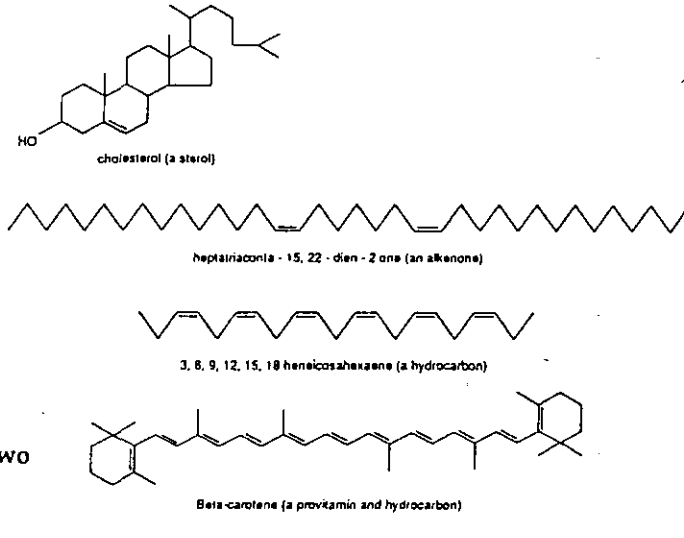

Fig. 3: Structures of lipid components of algae. A sterol, an alkenone and two hydrocarbons are shown.

Fatty acids

Fatty acids constitute a major proportion of the lipid fraction of algae, accounting for 20–40% of total lipid on a weight basis [34], although the value may sometimes be as high as 86% (e.g. for *Fragilaria* sp. [161]). Fatty acids occur predominantly in an esterified form with glycerol (see Fig. 2), and are found in tri- and di-glycerides, phospholipids and glycolipids. Most studies report only the total cell fatty acids, although some report the fatty-acid profiles of these lipid fractions [74, 182].

Data on the algae commonly used in mariculture are presented in Table 5. The different classes of algae can show quite distinct distribution patterns [11, 31, 161, 206, 207, 209, 219]. Saturated fatty acids constitute about 15–30% of the total fatty acids in green algae; the range in diatoms and prymnesiophytes is 30–40%. Green algae are low in the mono-unsaturates (5–20%) but high in the polyunsaturates (50–80%), whereas prymnesiophytes and diatoms have similar levels of both the mono-unsaturate (20–40%) and poly-unsaturate (20–50%) fractions. The polyunsaturate fraction of green algae, however, is dominated by 16 and 18 carbon-chain-length fatty acids, whereas levels of the higher carbon fatty acids (e.g. 20:5ω3 and 22:6ω3) are typically lower than those of other algal groups. Despite these similar trends, the levels of specific fatty acids may vary widely in closely related species in the same class. In particular, differences in the levels of the polyunsaturated fatty acids (PUFAs) 20:5ω3 and 22:6ω3 are important in the nutrition of maricultured animals (see chapter 3).

Table 5: **Fatty-acid composition (g/100 g of total fatty acid fraction) of some microalgae commonly used in mariculture.**
All values are from algae harvested during exponential growth phase.
TR = trace amount detected. – = not detected

	Algal species								
Fatty acid component	*Isochrysis galbana*	*Isochrysis* sp. clone T-ISO	*Pavlova lutheri*	*Chaetoceros calcitrans*	*Phaeodactylum tricornutum*	*Skeletonema costatum*	*Thalassiosira pseudonana*	*Dunaliella salina*	*Tetraselmis suecica*
saturates	37.0	32.2	35.9	30.2	30.2	39.2	27.2	23.3	26.8
monounsaturates	30.4	26.1	20.4	33.8	35.3	32.0	19.5	24.0	20.5
C16 polyunsaturates	0.4	2.6	0.8	13.4	10.2	13.1	22.2	6.8	17.2
18:2ω6	2.3	2.5	1.5	0.8	1.1	2.2	0.4	10.9	13.9
18:3ω6	0.2	2.4	0.4	0.4	—	0.3	0.2		2.7
18:3ω3	0.4	3.6	1.8	TR	1.3	0.3	0.3	30.5	4.6
18:4ω3	8.0	17.4	6.0	0.5	1.3	2.2	5.3	—	4.8
18:5ω3	—	2.0	—	—	—	—	—	—	—
20:4ω6	0.1	—	TR	5.7	—	—	0.3	—	2.1
20:4ω3	—	—	—	0.2	—	TR	0.3	—	0.1
20:5ω3	7.2	0.2	19.7	11.1	14.7	6.0	19.3	—	5.3
22:5ω6	—	1.8	2.0	—	—	—	—	—	—
22:6ω3	4.3	8.3	9.4	0.8	0.3	2.0	3.9	—	TR
Reference:	[209]	[206]	[206]	[206]	[15]	[206]	[206]	[16]	[206]

Phospholipids

Phospholipid may constitute from 5–25% of total lipid weight, but averages about 10% in most species [16]. Phospholipid subfractions detected in most algae include phosphatidyl inositol, phosphatidyl choline, phosphatidyl glycerol, phosphatidyl ethanolamine and diphosphatidyl glycerol [16]. Phosphatidyl choline and phosphatidyl inositol are present in prymnesiophytes and green flagellates [16]. Cryptomonads contain only phosphatidyl choline [11], and blue-green algae lack both these components [151].

Sterols

Sterols (Fig. 3) are minor components of the lipid fraction (0.5–2.5%; [9, 161]). Marked variations between the total sterol content and the type of sterols present have been noted in a number of marine algae [9, 137, 152, 161, 207]. The sterol content of three algae are given in Table 6.

Table 6: Sterol composition of selected microalgae (mg/g dry wt.) taken from [137].
— = not detected.

	Algal species		
Sterol	*Isochrysis galbana*	*Pavlova lutheri*	*Tetraselmis suecica*
cholesterol	0.02	0.01	0.01
24-methylene cholesterol	0.01	—	0.54
campesterol/24 epicampesterol	0.01	1.31	0.51
brassicasterol/24 epibrassicasterol	2.35	—	—
sitosterol/clionasterol	0.03	5.84	—
stigmasterol/poriferasterol	—	0.81	—
Total	2.42	7.97	1.06

Hydrocarbons and alkenones

Hydrocarbons and alkenones are two other classes of lipids that may be of nutritional importance in algae [16, 141, 199, 203, 204]. Saturated or mono-unsaturated hydrocarbons constitute a minor proportion (e.g. 0.1–2%) of the total lipid component of most algae, although there are exceptions such as the halotolerant algae *Botryococcus braunii*, where levels may reach 15% of the total lipid fraction [15]. A specific lipid fraction enriched in both alkenones and cyclic and polyunsaturated hydrocarbons has also been reported for a number of algae [15, 18, 141].

Pigments

The major pigments of most algae are the green chlorophylls and the yellow, orange and red carotenoids, which contribute 0.5–5% of the dry weight of the cell [16, 164]. Blue-green algae, red algae and the cryptomonads also contain the red, protein-bound water-soluble phycoerythrins and/or the blue phycocyanins. Chlorophylls and carotenoids are contained in the extracted lipid fraction of the cell. Carotenoids (Fig. 3), made up of a number of isoprene units, function both as photoprotectants and light-harvesting pigments in photosynthesis [34]. Each algal species may contain between 5 and 10 different carotenoids; over 60 different carotenoids are known to occur throughout the algal phyla [34].

ß-carotene, or provitamin A, is a common constituent of the carotenoid fraction of algae. It is found in highest concentration in the green algal classes. Although it generally constitutes less than 1% dry weight, it may accumulate to levels of up to 10% dry weight in halotolerant algae such as *Dunaliella bardawil* and *Dunaliella salina* [16, 74].

Chlorophyll *a* is the primary photosynthetic pigment in all algae. The accessory chlorophyll *b* is found together with chlorophyll *a* in the green algae, whereas chlorophyll *c* is found with chlorophyll *a* in the brown chromophyte algal classes (see Table 7).

Table 7: The major light harvesting-pigments of marine microalgae (updated from Jeffrey [100]).
* Fucoxanthin is found in some dinoflagellates containing chrysophyte-like endosymbionts.
† Fucoxanthin derivatives, 19'-hex- and 19'-butanoyloxyfucoxanthin are found in some species.
§ Two groups of prasinophytes contain a chlorophyll *c*-like pigment.
+ = present; – = not detected
The parentheses indicate where algal species with different pigment profiles exist within the same algal class.

		Major accessory light-harvesting pigments								
		Chlorophylls						Biliproteins		Carotenoids
Division	Common name	a	b	c_1	c_2	c_3	other§	Phycoerythrin	Phycocyanin	
Procaryote										
Cyanophyta	blue-green algae (cyanobacteria)	+						+	+	zeaxanthin
Eucaryote										
Rhodophyta	red algae	+						+		
Cryptophyta	cryptomonads	+			+			+	+	alloxanthin
Dinophyta	dinoflagellates	+			+					peridinin*
Chrysophyta	golden-brown flagellates	+		—	+	+				
	silicoflagellates	+		+	+					fucoxanthin†
Raphidophyta	raphidophyte	+		+	+					fucoxanthin
Prymnesiophyta	prymnesiophyte	+		—	+	+				
		+		+	+	—				fucoxanthin†
Bacillariophyta	diatoms	+		+	+	—				fucoxanthin
		+		—	+	+				
Euglenophyta	euglenoids	+	+							
Chlorophyta	green flagellates	+	+							lutein
	prasinophytes	+	+							lutein
		+	+				+			prasinoxanthin
		+	+				+			siphonaxanthin

Nucleic acids

The nucleic acid fraction of the algal cell can vary from 1–10% of the dry weight, although the usual range is 4–6%. Few investigators have studied qualitative differences in the nucleic acids of different algae; the ratio of RNA:DNA is approximately 3:1 [13, 91, 172]. When algae are grown in a high nutrient (e.g. nitrate) medium, the ratio of RNA:DNA may be as high as 50–200:1 [65, 67].

Minerals

The mineral fraction of the algal cell can constitute a major proportion of the dry weight, ranging from 6–39% (Table 2), but there are few detailed analyses. Algae can be a major source of a number of minerals. They can also accumulate trace- and heavy-metal ions, which can be a disadvantage if the metals are toxic [63, 70, 102, 174]. Major ions of known biological importance found in algae include phosphorous, silica (diatoms), calcium, sodium, potassium, chlorine, iron, magnesium and zinc; manganese, copper and cobalt occur in trace amounts. The elemental composition of two algal species are shown in Table 8.

Vitamins

Algae are a significant source of nearly all the vitamins. They have been studied in freshwater microalgae [2, 13, 19] and marine macroalgae [96, 97] but seldom in marine microalgae [1, 55, 107]. The major vitamins identified are thiamin (vitamin B1), riboflavin (B2), pyridoxine (B6), cyanocobalamin (B12), biotin, ascorbic acid (C), nicotinic acid, pantothenic acid, choline, inositol, tocopherol (E) and ß-carotene (provitamin A)

[see Table 9]. Vitamin K has been detected in trace amounts in *Porphyridium cruentum* when grown heterotrophically [6]. Vitamin D precursors have also been isolated from algae [95]. Many algae require vitamins, particularly thiamin, cyanocobalamin and biotin [169].

Table 8: **Mineral composition in two marine microalgae, given as mg/g dry weight. Data from Fabregas and Herrero [63].**

	Algal species	
Elements	*Isochrysis galbana*	*Tetraselmis suecica*
Ca	16.2	20.8
P	10.2	6.5
Na	7.2	10.4
K	5.6	12.0
Cl	50.8	37.2
Fe	3.6	1.0
Mg	11.5	7.8
Zn	0.6	1.5
Mn	0.04	0.05
Co	0.01	0.005
Cu	0.2	0.6

Table 9: **Major vitamin content of different marine microalgae (µg/g dry weight). R = vitamin required for growth; n.d. = not determined. The data are from a table compiled by Borowitzka [19].**

	Algal species		
Vitamin	*Ochromonas danica*	*Chaetoceros simplex*	*Peridinium cinctum*
cyanocobalamin (vitamin B_{12})	0	0.05	0.2
pyridoxine (vitamin B_6)	23	1.8	1–3
riboflavin (vitamin B_2)	35	5.3	26.6
thiamin (vitamin B_1)	R	3.2	2–9
pteroylmonoglutamic acid	9	2.1	0.4–0.7
biotin	R	1.8	0.2–0.3
nicotinic acid	89	62.3	9–18
pantothenic acid	37	29.5	7
ascorbic acid (vitamin C)	830	n.d.	n.d.
tocopherol (vitamin E)	2170	n.d.	n.d.
ß-carotene (provitamin A)	137	n.d.	n.d.
Reference:	[1]	[107]	[55]

3 Nutritional Requirements of Animals

Gross Composition

Protein

Protein fulfills an animal's need for nitrogen and essential amino acids; the amount required is influenced by genetic, environmental and nutritional factors. The age of the animal is also important, because as an animal's growth slows, its metabolic rates and protein requirements also decrease. Thus larval and juvenile animals have a greater protein requirement than adults. The levels of the essential amino acids are important. Also, all dietary components interact on a metabolic level and influence the utilisation of protein. For example, carbohydrate and lipids can be catabolised for energy and thus "spare" protein [196].

Larval molluscs require 30–60% (dry weight) of protein in their algal diet for good growth. However, there is not a clear correlation between protein content (expressed as % dry weight of alga) and nutritional value. For example, *Dunaliella salina* has a higher proportion of protein than *Chaetoceros calcitrans* (Table 2), but is of inferior value when eaten on its own. Webb and Chu [219] suggested that protein concentration (i.e. amount of protein/unit cell volume) is a better measure than protein expressed as a percentage of cell dry weight, and that a high concentration of protein in an alga is related to satisfactory food quality. However, the results of other investigations suggest correlation is poor (Table 10). *Isochrysis galbana* and *Pavlova lutheri*, both considered good food for molluscs, have well below average protein concentration, whereas *Dunaliella tertiolecta* (high protein value) used singly is a poor food for a number of molluscs. Clearly other nutrients are important; mixed algal diets are therefore more likely to provide all the nutrients required by the maricultured animal.

Table 10: **Protein concentration (expressed as mass/unit cell volume) for different algal species.**

Algal species	Cell volume (μm^3)*	[Protein] ($fg/\mu m^3$)†	Reference
Cryptomonas maculata	395	805	[147]
Dunaliella tertiolecta	230	143	[147]
Chaetoceros sp.	35	129	[25]
Pyramimonas virginica	34	61	[219]
Nannochloris oculata	6	55	[219]
Chlorella sp.	5	39	[219]
Tetraselmis suecica	390	26	[219]
Skeletonema costatum	402	24	[147]
	350	63	[25]
Isochrysis galbana	58	9	[219]
	31	258	[147]
Pavlova lutheri	74	5	[219]

* $1\ \mu m^3 = 10^{-15}$ litres

† $1 fg = 10^{-15} g$; therefore the ratio $fg/\mu m^3$ is equivalent to both g/litre and mg/ml

For crustaceans, algae with protein concentrations in the range 30–60% (of dry weight) have been used successfully as food for early prawn larvae [116, 134]. The protein requirements of various life stages of prawn species has been established by artificial diets [24, 110, 196] as 30–50% (dry weight).

Fish require 40–60% protein in their diet [24, 38, 41, 155, 175]. The specific requirement for protein depends on the habitat (freshwater, estuarine, marine) and whether the animal is omnivorous, herbivorous or carnivorous [5, 77, 162].

Carbohydrate

For bivalve molluscs, a tentative correlation has been made between the total algal carbohydrate content and nutritional value [72, 86], although carbohydrate may be 5–30% of the dry weight of algae that have been found to support good growth (Tables 1 and 2). Enright *et al.* [56] attributed the high ranking of the alga *Rhodomonas* sp. as food for juvenile *Ostrea edulis* to its high carbohydrate level per cell. These and other workers have noted, however, that high-quality diets must also have high levels of PUFAs 22:6ω3 and 20:5ω3 [27, 56, 88, 209].

Optimum levels of dietary carbohydrate for prawn (*P. japonicus*) larvae have been established at 15–25% of the dry weight of the diet [196]. Similar values have been advocated for most fish [38].

In all animals, the specific requirement for carbohydrate is influenced by genetic, environmental and nutritional considerations, as outlined in the previous section. In particular, the importance of carbohydrate in sparing dietary protein has been noted. The influence of the "quality" of the carbohydrate fraction is discussed in a later section.

Lipids

Dietary lipids are sources of both metabolic energy [93, 94] and specific metabolites that are essential for animal growth (i.e. fatty acids, phospholipids, sterols, hydrocarbons and alkenones). Waldock and Nascimento [209] showed that differences in the growth rates of *Crassostrea gigas* larvae were not correlated with the amount of total lipid in the algal diet (which can range from 5–23%; see Table 2), although the largest larvae contained the greatest percentage (by weight) of triglyceride.

Diets on which crustaceans and fish show satisfactory growth generally have between 10–20% lipid [24, 38, 166]. However, high dietary lipid results in high levels of lipid being deposited in the body of the animal and discarded as visceral fat during processing [24].

Effect of algal composition on zooplankton

As most species of fish larvae feed on "algae-fed" zooplankton, the effect of the gross biochemical composition of the algae on the nutrition of fish is less direct.

The gross compositions of the rotifer (*Brachionus plicatilis*) [15] and *Artemia salina* larvae [33] are closely related to the gross compositions of their algal diets [15]. There was little difference in the growth and gross composition of rotifers fed on different species of algae (except that rotifers fed *Dunaliella tertiolecta* were richer in carbohydrate), although the gross composition of the algae was not assessed in this study [178].

Both the temperature and feeding ration under which the zooplankter is cultured can also change its biochemical composition. At low temperatures, rotifers accumulate more carbohydrate and lipid [178].

Essential Components

Apart from nutritional requirements for protein, carbohydrate and lipid, animals have specific requirements for nutrients within these fractions. These requirements (in addition to mineral requirements) are outlined in the following sections.

Amino Acids

A number of amino acids are "essential" for maricultured species (Table 11; see also [39, 85, 112]). In this review an "essential" nutrient is defined as one that cannot be synthesised in sufficient quantities in the body to meet growth requirements, but must be provided in the diet. Some amino acids, although not strictly essential, may be important in sparing essential amino acids. For example, cysteine can be made from methionine (an essential amino acid), but provision of adequate cysteine in the diet will reduce the dietary need for methionine. Similarly, tyrosine in the diet will reduce the dietary requirement for phenylalanine.

Table 11: Essential amino acids for maricultured species and the amino-acid composition (expressed as g/100 g of total amino acid in hydrolysate) of various animals compared to the range present in algae. As proline is not an essential amino acid for crustaceans and fish, proline values for these animals are given in parentheses. Tyrosine and cysteine, although not essential amino acids, may be nutritionally important in "sparing" dietary phenylalanine and methionine, respectively. n.d. = not determined. * (e.g. juvenile mussel [85]) † (e.g. prawn larvae [197]) § (e.g. sole egg-yolk [45])

Amino acid	Bivalves*	Crustaceans†	Fish§	Range of composition in algae (from Table 3)
Threonine	6.9	3.8	5.0	3.6 – 6.2
Valine	5.0	5.6	5.9	4.2 – 7.1
Methionine	2.4	3.7	1.8	1.6 – 3.2
Isoleucine	4.0	5.9	6.3	2.9 – 5.1
Leucine	7.7	7.8	8.0	6.7 – 10.2
Phenylalanine	3.7	5.6	4.6	2.8 – 6.0
Lysine	4.8	8.4	7.7	5.1 – 12.0
Histidine	1.3	2.9	3.4	1.4 – 3.6
Arginine	5.9	9.1	6.5	5.7 – 11.3
Tryptophan	0.4	4.1	n.d.	0.0 – 1.7
Proline	1.5	(6.6)	(10.9)	3.2 – 6.7

Generally, it has been found that dietary protein with an essential amino acid pattern similar to that of animal whole body or egg proteins (see Table 11) have high nutritive value for that animal [7, 47]. Hence protein sources may be ranked in terms of an adequacy index. For algae, this is defined as the percentage composition of the essential amino acid in an alga, divided by the composition of the same amino acid in the body tissue of the feeding animal, multiplied by one hundred. The index was calculated for a number of algae of potential food value for the mussel *Mytilis californianus*, and algae were ranked in descending order of predicted protein quality [219]. Some correlation was found between the indices of the limiting amino acids and reported

food values (e.g. [213]). *Tetraselmis suecica, Pavlova lutheri* and *Isochrysis galbana* were ranked high in the index (and supported growth well) whereas *Chlorella* sp. and *Phaeodactylum tricornutum* were ranked low (and supported little or no growth).

Whether the levels of essential amino acids are a major factor in determining the overall nutritional value of an alga is debatable. Minor variations observed in the amino-acid composition of algae do not correlate with often large differences in their ability to support growth of a particular animal. There appears to be no strong evidence that a deficiency of a specific amino acid in any alga results in its being an unsatisfactory food for an animal. *Phaeodactylum tricornutum*, which is reported as totally lacking in tryptophan [61], does not support the growth of juvenile *Ostrea edulis* [56] or juvenile *Crassostrea virginica* [61]. However, other algae reported to be low in tryptophan have high as well as low food value [32, 35, 56, 61].

Different algae may produce only minor variations in the amino-acid composition of the zooplankton that feed on them. Thus *Artemia salina* fed either *Spirulina* sp. or *Scenedesmus* sp. has an almost identical amino acid composition, although the low methionine levels in *Scenedesmus* sp. caused an amino acid deficiency in *Artemia* sp. fed with this alga [33]. The authors of this study acknowledge, however, that the results may not be valid since some of the methionine may have been destroyed by hydrolysis.

Amino-acid analysis in algae requires very careful techniques. When a crude protein sample, such as an algal cell, is subjected to acid hydrolysis, materials such as lipids, carbohydrates and minerals can interfere, leading to poor recoveries and an underestimation of certain amino acids, particularly cysteine, methionine and tryptophan. Workers commonly prepare hydrolysates with 6M HC1, a reagent that can cause complete or partial destruction of tryptophan [90]. However, alternative reagents that give a much greater recovery of this amino acid [185] are recommended.

In summary, the importance of the amino-acid composition of algae used as food in mariculture is still unclear, and more studies need to be done to evaluate their role.

Carbohydrate quality

Although the contribution of the total carbohydrate in the algal diet of maricultured animals has been well studied, relatively few reports have addressed the role of carbohydrate quality, i.e. the relative proportions and availability of the individual sugars making up the carbohydrate fraction.

Carbohydrate composition, in terms of specific sugars, may vary considerably in algal species (Table 4). Glucose generally has the highest concentration, followed by galactose, mannose and ribose. While relative proportions may be significant, the form of the sugar is equally important. Sugars may occur as mono-saccharides, di-saccharides and simple oligo-saccharides, or polysaccharides. The class and specific links within a polysaccharide will determine whether the component sugars are readily digested by the feeding animal.

The method of sugar analysis is very important when correlating chemically measured carbohydrate values and the nutritional value of algae. Most analyses do not differentiate the form of the component

sugars, and therefore fail to establish what sugars would be assimilated by the feeding animal.

Polysaccharides such as cellulose or chitin, which are chemically stable and therefore not accounted for in the methods for total carbohydrate analyses presently used, may themselves be digested (e.g. by the enzymes cellobiase or chitobiase in the oyster *C. virginica* [145]).

Parsons *et al.* [164] suggested that carbohydrate composition was a substantial factor determining the nutritional value of an alga, and postulated that the high content of glucose in both *Pavolva lutheri* and *Skeletonema costatum* made them a satisfactory food for various organisms. However, this correlation has been refuted by Webb and Chu [219], who point out that other algal diets with similar glucose levels supported different levels of growth of oyster larvae. For example, *Chaetoceros* sp., found to be an excellent food for spat and larvae of *Crassostrea gigas* [209, 213, 214], has a low to intermediate level of glucose compared to other algae.

The qualitative differences in the class of polysaccharides in different algae have already been noted. It has been suggested that amylase and laminarase enzymes in the digestive system of bivalves would account for the efficient breakdown and assimilation of all the mannan and glucan polysaccharides [221].

The importance of the particular form of dietary carbohydrate has been demonstrated for crustaceans. Abdel-Rahman *et al.* [3] showed that juveniles of the prawn *Penaeus japonicus* grew far better on artificial diets containing disaccharides and polysaccharides than on diets containing monosaccharides. Monosaccharides such as glucose are quickly absorbed from the stomach and released all at once into the haemolymph, resulting in abnormally high levels of blood glucose. However, dissaccharides and polysaccharides are slowly digested, leading to a gradual release of monosaccharides into the haemolymph and more efficient utilisation of the energy source.

Carbohydrate type may also be critical in fish diets. Degani *et al.* [44] fed groups of European eel (*Anguilla anguilla L.*) on artificial isonitrogenous diets containing carbohydrate from different sources. They found marked differences in the growth rates of the groups; for example eels fed a diet with wheat meal as the carbohydrate source grew four times faster than eels fed a diet containing potato starch. Differences in growth rate were attributed to different degrees of utilisation of the carbohydrate sources.

In summary, the limited data available suggest that carbohydrate quality is of some importance. One way to ascertain the degree of importance would be to extract carbohydrate fractions from different algae, incorporate these at fixed rations into appropriate artificial feeds, and conduct feeding trials.

Lipid fractions

The most important aspect of lipids in animal nutrition is the content and proportions of the algal fatty acids.

Bivalve molluscs For the spat of bivalve molluscs, two PUFAs (20:5ω3 and 22:6ω3) have been shown to be essential [131]. *Dunaliella tertiolecta* (which contains no PUFA of chain length greater that C18) fed to *Crassostrea gigas* spat did not sustain growth, although growth was

observed when the diet was supplemented with microencapsulated 22:6ω3. In the same experiments, growth was satisfactory with *Tetraselmis suecica*, an alga deficient in 22:6ω3 but containing 20:5ω3. These results indicated that the PUFAs of the ω3 family were essential, but either 20:5ω3 or 22:6ω3 was adequate. Other bivalve molluscs may have similar requirements. Algae lacking these fatty acids are unsatisfactory foods; algae that contain significant levels of at least one component are satisfactory [56, 89, 126, 167, 206, 213]. The main physiological role of the PUFAs, after incorporation into phopholipids, appears to be to maintain membrane integrity and permeability [28].

It has been shown, at least in *Crassostrea virginica*, that these PUFAs are not detectable in newly hatched larvae, but accumulate during feeding and normal growth [28]. Either they are derived from the diet or older larvae may be able to biosynthesise ω3 fatty acids *de novo* (or possibly both). The yellow clam *Mesoderma matroides* can elongate and desaturate linoleic acid (18:2ω6) and α-linolenic acid (18:3ω3) from dietary phytoplankton [46]. Also, when isotopically labelled *Dunaliella tertiolecta* (containing fatty acids no longer than C18) and *Tetraselmis suecica* (containing no 22:6ω3) were fed to oysters (*C. gigas*), PUFAs absent from the diet were detected in low levels in the oyster tissue [208]. It appears that juvenile oysters may be able to elongate and desaturate dietary precursors (*de novo* synthesis), but at too low a level to sustain growth. Even if a significant conversion from precursors (such as linolenic acid) were possible, this biosynthesis would require input of additional energy. Therefore growth would still be enhanced if these PUFAs were included in the diet.

Algal diets that support satisfactory animal growth have cellular concentrations (mass per unit cell volume) of the PUFAs 20:6ω3 and 22:6ω3 ranging between 1–20 fg/μm^3 (note: 1 fg/μm^3 = 1mg/ml) (Table 12). Algae with PUFA concentrations lower than 0.5 fg/μm^3 are often associated with poor animal growth when fed as a single species. However, the minimum levels of PUFA for different species or different growth stages of the same species are not known.

The role of other dietary shorter-chained PUFAs in nutrition (e.g. linoleic acid (18:2ω6) and a-linolenic acid (18:3ω3)) is less well defined. The ω6 class of PUFAs may be required by oyster larvae [219].

Table 12: Growth response of larval and juvenile bivalve molluscs fed with algae containing various concentrations of the PUFAs 20:5ω3 + 22:6 ω3

* Data recalculated from Volkman *et al.* [206].

† Taken from Table 1.

§ Note 1 fg/μm^3=1 mg/ml

Algal species	[20:5ω3 + 22:6ω3]/cell* (fg/μm^3)§	*Number of reports of alga supporting growth*†	
		satisfactory	poor
Chaetoceros calcitrans	17.8	11	0
Pavlova lutheri	10.1	8	3
Thalassiosira pseudonana	7.2	6	0
Chroomonas salina	3.9	1	0
Chaetoceros gracilis	3.2	1	0
Isochrysis sp. clone T-ISO	2.0	8	2
Skeletonema costatum	0.8	3	1
Nannochloris atomus	0.3	0	2
Tetraselmis suecica	0.2	6	3
Dunaliella tertiolecta	0.0	0	6

Crustacea The requirements and metabolism of fatty acids in crustaceans have been reviewed by Castell [23]. The requirements of the different species of crustaceans are not uniform, but 18:2ω6, 18:3ω3, 20:5ω3 and 22:6ω3 may all be important. Kanazawa and Teshima have

shown that a number of juvenile and adult *Penaeus* spp. do not synthesise PUFAs *de novo* [111, 114, 115]. Although prawns can elongate and desaturate linoleic (18:2ω6) and a-linolenic (18:3ω3) acids, they cannot produce sufficient quantities of the long chain ω3 PUFAs to maintain maximum growth.

Larvae of *Penaeus japonicus* have similar requirements [103]. Prawn larvae fed diets containing either radiolabelled palmitic acid (16:0) or of a known fatty-acid composition showed no ability to biosynthesise 18:2ω6 and 18:3ω3. In addition, the results suggested that the rates of conversion of 18:2ω6 to 20:4ω6 and 18:3ω3 to 20:5ω3 and 22:6ω3 were extremely low. Growth was more efficiently promoted by 22:6ω3 than by 18:3ω3, and was further enhanced by diets containing 20:5ω3 (in addition to the other PUFAs mentioned). The authors suggest that prawn larvae and juveniles have the same metabolic pathways with respect to fatty-acid desaturation and elongation, with some differences in relative activities, and that both life stages require the w3 fatty acids, particularly 20:5ω3 and 22:6ω3. The optimal dietary levels (defined from studies using artificial diets) of these PUFAs appears to be about 1% [119, 120].

Other crustaceans can elongate and desaturate fatty acids of the linolenic and linoleic series. Brine shrimps (*Artemia salina*) can synthesise considerable quantities of 20:5ω3 when fed on *Chaetoceros simplex*, a diatom containing 18:3ω3 but deficient in 20:5ω3 [121].

The importance or essentiality of the ω6 PUFAs, in particular 18:2ω6, is not clear, although some evidence suggests that the amount in the diet may be important for some species. One study showed that when both 18:2ω6 and 18:3ω3 were added to the diet of the prawn *Palaemon serratus*, they produced better growth than either PUFA alone [142]. The levels of 20:3ω6 are higher in the gonad of the horseshoe crab *Xiphosura polyphemus* than in other tissues [202]. This fatty acid, presumably derived from ω6 dietary precursors, may be an essential precursor for prostaglandin biosynthesis, therefore playing an important role in the physiology of the crustacean reproductive organ [23].

Fish The essential fatty acid requirements of fish are apparently determined by their environment, their feeding habits and their position in the food chain [38, 39]. Cold-water fish generally require more PUFAs than warm-water fish to maintain the fluidity of the cell membranes at low temperatures [202].

The natural diet of carnivorous fish probably contains an excess of PUFAs. These fish may be less able than herbivorous fish to convert fatty acids and therefore require more essential fatty acids.

Salmon, carp and eel larvae require a certain proportion of linoleic and linolenic acid to achieve maximum growth [42] and readily convert linolenic acid to 20:5ω3 and 22:6ω3 [22, 227]. However, not all marine fish, including the larvae of Red Sea bream (*Chrysophyrs major*) [42] and of turbot (*Scophthalmus maximus*) [179] have this capacity. Marine fish larvae respond much better to the PUFAs 20:5ω3 and 22:6ω3 than to 18:3ω3 and 18:2ω6 [42]. From artificial diets, it has been established that the PUFA requirement for a number of fish species is in the range of 1–2% (of dry weight) [24].

The nutritional role of the ω6 PUFAs in marine fish, as in crustaceans and bivalve molluscs, is not well understood, although some freshwater species require these PUFAs [37, 113, 114].

Zooplankton The levels of the PUFAs in many of the zooplankton commonly fed to fish and crustacean larvae are influenced by their algal diet. Rotifers raised on *Dunaliella tertiolecta* (deficient in 20:5ω3 and 22:6ω3) are low in these PUFAs. In contrast, rotifers raised on *Pavlova lutheri*, an alga rich in ω3 PUFAs, also become rich in the ω3 PUFAs [179]. Similar findings have been reported for *Artemia salina* fed diets either low or high in the PUFAs [210, 218]. On the other hand, the less easily cultured copepods (*Tigriopus* sp. and *Acartia* sp.) are naturally high in levels of 20:5ω3 and 22:6ω3 and are obviously capable of significant *de novo* biosynthesis. The levels of PUFAs in these species are therefore less influenced by diet [217, 218].

Phospholipids

In addition to specific requirements for choline, inositol and PUFAs, intact phospholipids are essential for some animals. The inclusion in artificial diets of phospholipids with either an inositol or choline group (at 1% of dry weight of the diet) was essential for the growth and survival of prawn (*P. japonicus*) larvae [108, 109, 195]. However, juvenile lobsters (*Homarus americanus*) do not require dietary phospholipid [122]. Possibly prawns require them for the transport of dietary lipids (particularly cholesterol) in the haemolymph, as their rate of phospholipid biosynthesis is slow.

Sterols

Sterols are important as membrane constituents and as precursors of bile salts, bile acids and steroid hormones [36].

In bivalve molluscs, *de novo* biosynthesis of sterols has been indicated in some animals (e.g. for *Mytilus californianus* [68] and *Mytilus edulis* [194]). However, the consensus is that bivalve molluscs have a limited capacity for sterol biosynthesis, and require dietary sources of sterols for growth and survival, although optimal amounts have not been determined [192].

Larval, juvenile and adult crustaceans require dietary sources of sterols for normal development because they cannot biosynthesise sterols *de novo* [40, 192, 193]. Some species of prawns, crabs and lobsters can metabolise cholesterol to cholesteryl esters, steroid hormones and moulting hormones (ecdysone and ecdysterone) [192]. Cholesterol is often not the major sterol of live food organisms used in crustacean culture (including algae; Table 6).

The growth of animals fed on food containing other sterols (e.g. ergosterol, stigmasterol and ß-sitosterol) was inferior to that of animals fed on food containing cholesterol [192]. Prawn (*P. japonicus*) larvae [110] and juvenile crayfish (*Pacifastacus leniusculus*) [40] attained optimal growth when cholesterol was included in the diet at 0.5–1.0% dry weight; juvenile lobster required 0.2–0.5% [122].

Fish are capable of *de novo* synthesis of sterols [36] and therefore would have no absolute dietary requirements for them. However, as sterol levels (especially cholesterol) in tissues reflect a balance between dietary sterol and sterols biosynthesised *de novo*, the levels of sterols in the diet may well influence the health of fish.

Copepods and brine shrimps (both crustaceans) also require dietary sterols, which are supplied by algal-based diets. Although algae contain different proportions of sterols, the bulk of this sterol fraction can be converted by the animal to cholesterol and demosterol [168].

Hydrocarbons and alkenones

Hydrocarbons and alkenones (in addition to the ω3 PUFAs) may promote proper larval development by acting as anti-oxidants of PUFAs [15]. These suggestions are yet to be substantiated by experiment, but clearly are areas for future investigation.

In addition to these lipids occuring in varying amounts in algae, zooplankton (rotifers and brine shrimp) fed algal diets rich in hydrocarbons and alkenones will also accumulate high levels of these fractions [15].

Pigments

ß-carotene (a pro-vitamin A) has already been mentioned as essential for crustaceans. As a precursor of vitamin A it presumably would also contribute to the nutrition of fish and bivalves, though little work has been done on this aspect.

Dietary xanthophylls from algae are incorporated into the exoskeleton of prawns and lobster (as astaxanthin) and the flesh of salmonids (as canthaxanthin and astaxanthin) [34]. These compounds play a major role in pigmentation, but other functions are poorly defined.

The metabolism of carotenoids in animals is discussed in detail by Goodwin [80].

Chlorophylls may contribute magnesium to animal nutrition. These pigments are catabolised to phaeophorbides by the removal of magnesium in acid conditions and by the enzymatic removal of the phytol ester. The role that chlorophylls or their degradation products play in animal nutrition, other than contributing nitrogen and carbon, is yet to be determined.

Nucleic acids

No nutritional correlation between nucleic acid quality or quantity in algae has been advanced, although the nucleic acid is 4–6% of the algal cell dry weight and is therefore a significant component. Complete digestion of nucleic acids yields phosphate, ribose (a sugar) and pyrimidines and purines, which the feeding animal may use to synthesise its own nucleic acids, thus sparing its own *de novo* synthesis.

Excess dietary purines and pyrimidines may be oxidised. Marine animals convert waste nitrogen to more soluble forms (e.g. allantoic acid, allantoin, urea or ammonia [220]). Catabolism of pyrimidines may yield free ammonia in addition to amino acids and fatty acid precursors, which are used by the animal.

Minerals

The contribution of algae to the mineral requirements of animals is difficult to establish. There are very few detailed analyses of the elemental composition of algae [63, 133] and only the requirements of cultured prawn (*P. japonicus*) larvae [108] and some fish species [24] have been reported. The minerals that have demonstrable biological functions in all animals, either in unbound (elemental) form or incorporated into specific compounds, are calcium, phosphorous, sodium, magnesium, potassium, sulphur, chlorine, cobalt, iron, copper, iodine, zinc, fluorine, molybdenum, manganese and selenium [154].

Animals may take up some proportion of the minerals directly in soluble form from seawater (see [190] for elemental composition of seawater), thus complicating the interpretation of the role of algae in supplying minerals.

There are conflicting reports on prawns' requirements for calcium and magnesium. It was reported that *Penaeus japonicus* takes up calcium from seawater and does not require dietary calcium, magnesium and iron, although it does require dietary supplementation of phosphorous, potassium and trace metals [50, 51]. However, Kanazawa *et al.* [117] demonstrated that this species did require calcium, phosphorous, potassium, magnesium and copper and recommended that the calcium requirement of prawns should be re-evaluated [110].

Marine fish constantly drink small amounts of water, which provides many of the cations required for metabolism [191]. Freshwater and marine fish species are reported to require calcium, phosphate, magnesium, zinc, manganese and copper [8, 124, 156, 157, 158, 159, 160]. Fabregas and Herrero recommended that dry, powdered algae be incorporated in the pellet feed of both freshwater and marine fish: up to 33% for freshwater fish would supply all their mineral needs, except phosphorous; and up to 50% for marine fish would supply all their mineral needs, except for manganese and cobalt [63].

The mineral composition of diets may also affect the absorption of other dietary nutrients. Fish larvae concentrate heavy metals in the intestine [101], reducing the absorption of essential amino acids.

Vitamins

The vitamin requirements of bivalve molluscs are uncertain. Some information has come from feeding experiments with totally artificial (i.e. no algae) diets where the precise chemical composition is controlled by adding specific amounts of the B group vitamins (i.e. thiamin, riboflavin, pyridoxine, cyanocobalamin, biotin, nicotinic acid, pteroylmonoglutamic acid and pantothenic acid), other water-soluble vitamins (ascorbic acid, choline and inositol) and the fat-soluble vitamins A, D, E and K [129, 130; Table 12]. The optimum levels of these vitamins have not been reported.

The vitamin requirements of both juvenile and larval prawns are well defined. Juvenile *Penaeus japonicus* require dietary thiamin, pyridoxine, ascorbic acid, choline and inositol [48, 49, 81, 118] (quantities are given in [108]). Larval *P. japonicus* require thiamin, riboflavin, pyridoxine, cyanocobalamin, nicotinic acid, biotin, pteroylmonoglutamic acid, ascorbic acid, inositol, choline, ß-carotene, and vitamins D and E (Table 13). Larvae may require more of some vitamins (e.g. ascorbic acid) than do juveniles [110].

The vitamin requirements of fish have been reviewed by Cowey and Sargent [36]. They differ markedly, even between some closely related fish species. It is often difficult to demonstrate a vitamin deficiency (i.e. establishing the essentiality of a specific vitamin) because vitamins obtained from the intestinal bacteria might mask the inability of the animal to synthesise the vitamin. Fish and prawns appear to have quite similar vitamin requirements (Table 12). However, no dietary requirement for vitamin D has been demonstrated in fish, and whilst fish require pantothenic acid and vitamin K, prawn larvae do not.

One neglected aspect is that a vitamin may be "bound" within the algal cell and not be available for assimilation by the feeding animal. Rotifers were unable to utilise bound cyanocobalamin when fed *Dunaliella* sp., but obtained their dietary needs from soluble cyanocobalamin excreted by the alga into the seawater [180].

Table 13: Qualitative vitamin requirements for maricultured species.
Quantitative vitamin requirements are given in [110] for crustaceans and in [24] for fish.
* Minimum range of vitamins required to grow larvae to metamorphosis.
n.r. = no dietary requirement demonstrated.

Bivalves†	Crustaceans§	Fish††
thiamin	thiamin	thiamin
riboflavin	riboflavin	riboflavin
pyridoxine	pryidoxine	pryidoxine
cyanocobalamin	cyanocobalamin	cyanocobalamin
biotin	biotin	biotin
nicotinic acid	nicotinic acid	nicotinic acid
pteroylmonoglutamic acid	pteroylmonoglutamic acid	pteroylmonoglutamic acid
pantothenic acid	n.r.	pantothenic acid
choline	choline	choline
inositol	inositol	inositol
ascorbic acid	ascorbic acid	ascorbic acid
n.r.	ß-carotene	n.r.
vitamin A	n.r.	vitamin A
vitamin D	vitamin D	n.r.
vitamin E	vitamin E	vitamin E
vitamin K	n.r.	vitamin K

† e.g. *Crassostrea virginica* larvae [30]
§ e.g. *Penaeus japonicus* larvae [110]
†† several species [36]

4 Other Aspects of the Use of Microalgae in Mariculture

Algal Culture

Methods of small-scale and mass culturing of microalgae are well documented [82, 99, 135]. An enriched seawater medium (Guillard's f/2) [82] is the most common: to the seawater are added nitrate, phosphate, silicate, EDTA, iron, copper, zinc, sulphate, cobalt, manganese, molybdenum, and the B group vitamins (thiamin, biotin and cyanocobalamin).

At the CSIRO Marine Laboratories, Hobart, Australia, stock cultures of algae are maintained in 75 ml of media in 125 ml Erlenmeyer flasks under a controlled environment (12h:12h light:dark cycles; irradiance of 70–80 $\mu E\ m^{-2}s^{-1}$; temperature 20–22°C). Cells in the exponential growth phase are transferred by aseptic techniques [135]. Stock cultures are transferred to another 125 ml flask (using 0.5–2.0 ml inoculum) and, if larger volumes are required, 10–50 ml of inoculum is transferred to 2 litres of culture medium in 4-litre flasks. Hatcheries scale up further by inoculating 200–500 ml of culture to carboys (e.g. 20 litre), and from carboys to plastic bags (e.g. 200–500 litre), tanks (e.g. 1000–5000 litre) or larger ponds. Harvested algae can be in a typical cell suspension (e.g. 10^6–10^7 cells/ml) or a wet-packed paste collected by mild centrifugation (e.g. approximately 10^{10} cells/g).

The typical scale-up procedure is shown in Fig. 4. In practice, the axenic (bacteria-free) status of the culture is difficult to maintain beyond the large-flask stage. Thereafter attempts are made to keep bacterial numbers down by means of, for example, filtering seawater through a 0.2 µm membrane and harvesting cells in the exponential phase of growth [135, 136]. After the small flask, the cultures must be aerated to supply sufficient CO_2 for algal growth and to keep cells in suspension.

Training of hatchery and other personnel in algal cultivation and aseptic techniques is available annually through FIRTA-funded workshops (see [98]).

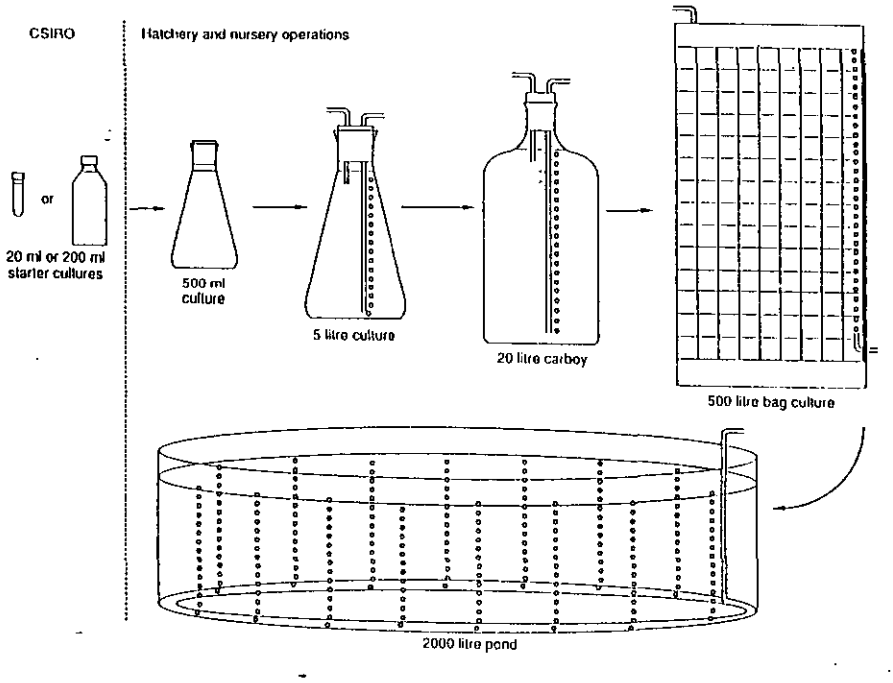

Fig. 4: Typical scale-up of microalgal production from CSIRO algal stocks to hatchery and nursery operation.

Mixed Algal Diets

Animals fed on more than one algal species grow faster than animals fed on a single algal species (Table 14; see also [43, 56, 126, 173]). This is because, while one alga may lack a particular nutrient, another alga may contain that nutrient and lack a different one. Hence, when both are fed to an animal, they supply an adequate amount of both micronutrients. Differences in algal digestibility may also contribute to this effect [60].

Table 14: Summary of algal diets shown to produce excellent growth in larval and juvenile animals. Diets are either uni-algal, or contain more than one alga.

Animal	Developmental Stage	
	Larval	Juvenile or adult
BIVALVE MOLLUSCS		
Oysters		
Crassostrea species	*Pavlova lutheri* + *Isochrysis galbana* [30] *Pyramimonas virginica* + *Pseudoisochrysis paradoxa* + *Chlorella sp.* [226] *C. calcitrans* [89] *I. galbana* (T-ISO) + *C. calcitrans* [89]	*Thalassiosira pseudonana* + *I. galbana* [173] *Chaetoceros calcitrans* [125] *T. pseudonana* + *I. galbana* + *Tetraselmis suecica* [58]
Ostrea species	*T. suecica* [225] *Chaetoceros gracilis* [56]126 *C calcitrans* + *T. suecica* [109] *C. calcitrans* + *Skeletonema costatum* [126] *T.suecica* + *S. costatum* [126]	*C. calcitrans* [56]
Scallops	*Isochrysis sp.* clone T-ISO [139] *P. lutheri* + *C. calcitrans* + *Rhodomonas baltica* [12]	*T. pseudonana* [59] *Isochrysis* sp. clone T-ISO + *C. calcitrans* + *T. pseudonana* [139]
Clams	*Isochrysis* sp. clone T-ISO + *C. calcitrans* [89] *C. calcitrans* [89] *Isochrysis* sp. clone T-ISO [89]	*C. calcitrans* [125] *S. costatum* [213] *I. galbana* + *T. pseudonana* + *T. suecica* [58]
Conch	*Prorocentrum minimum* [167]	
Mussel	*I. galbana* or *P. lutheri* [10]	*T. suecica* [213] *T. pseudonana* [59]
CRUSTACEANS		
Prawns	*C. gracilis* [116] *C. gracilis* + *Tetraselmis chuii* [134]	
ZOOPLANKTON		
Artemia	*I. galbana* [15, 222]	
Rotifers		*I. galbana* or *P. lutheri* or *P. tricornutum* [178] *Nannochloropsis salina* or *C. gracilis* [15]
Copepods	*I. galbana* + *Rhodomonas baltica* [189]	

Manipulation of Biochemical Composition of Algae

The biochemical composition of algae can be substantially altered by manipulating their culture conditions (Table 15), as the composition is dependent on the nutrient concentration and the composition of the growth medium [62, 64, 65, 66, 67, 132, 223], the temperature [79, 170], light intensity and wavelength [9, 69, 92, 132], photoperiod [148] and growth stage at time of harvest [149, 221].

Most studies have monitored variations in the gross biochemical composition, particularly differences between the exponential and stationary growth phases. As the algae's responses are species-dependent, generalisations can be misleading. However, lipid and/or carbohydrate levels typically increase [149, 163] and the protein levels decrease [163] during the stationary phase, due to nitrogen limitation.

Studies of changes in the levels of specific biochemical compounds are perhaps more important than those of gross composition. When nitrogen or silicate were limited, the concentration of 22:6ω3 in *Chaetoceros gracilis* was reduced [57]; when nitrogen was limited, the level of 20:5ω3 in *Isochrysis galbana* was reduced [16].

High photon flux densities or photoheterotrophic growth on glucose produces an increase in the degree of lipid unsaturation in the green alga *Scenedesmus* sp. [144]. In contrast, high temperatures decrease fatty-acid unsaturation in *Scenedesmus* sp. [144] and *Pavlova lutheri* [4].

Table 15: Specific documented effects of the environment on microalgal nutrients. ↓ = reduction, ↑ = increase, O = little or no change, Δcompn = change in composition.

Nutrient	Algae	Environmental Factor	Change	Reference
Protein	*T. pseudonana, I. galbana*	stationary phase	↓	[221]
	Nannochloris sp., *D. salina*, *Isochrysis* sp T-ISO	stationary phase	↓	[16]
	C. calcitrans, T. suecica	stationary phase	O	[221]
	Isochrysis sp. T-ISO	stationary phase	↑	[221]
Carbohydrate	*P. virginica, P. paradoxa, P. lutheri, I. galbana, Chlorella* sp.	stationary phase	↑	[29]
	T. pseudonana, I. galbana, T. suecica	stationary phase	↑	[221]
	D. salina, Nannochloris sp, Isochrysis sp T-ISO	stationary phase	↑	[16]
	S. costatum, C. socialis	stationary phase	↑	[22]
	Isochrysis sp. T-ISO	stationary phase	O	[221]
	C. calcitrans	stationary phase	↓	[221]
PUFAs	*Cyclotella meneghiniana*	photoperiod	↑ (during dark)	[184]
	D. salina	stationary phase	↑ 20:6	[16]
	Isochrysis sp. T-ISO	stationary phase	↓ 20:5ω3	[16]
	Scenedesmus sp.	high light intensity	↑	[144]
	P. lutheri	low temperature	↑	[4]
Sterol	*P. tricornutum*	stationary phase	↑	[9]
	D. minuta	stationary phase	↓	[9]
	D. minuta	light quality	Δcompn	[9]
Hydrocarbons	*Botryococcus braunii, Nannochloris* sp.	stationary phase	↑	[16]
ß-carotene	*D. salina*	high salinity	↑	[146]
	D. bardawil	stationary phase	↑	[14]
Vitamins	*Oscillataria javorensis, Chroococcus minutus*	stationary phase	↓ cyanocobalamin	[181]

The levels of other lipid fractions are also altered by culture conditions. The composition of sterols is influenced by illumination and the growth stage [9]. The neutral hydrocarbon content in *Botryococcus braunii* [16] and ß-carotene in *Dunaliella bardawil* [14] increases when nitrate is deficient. An increase in salinity increases production of ß-carotene in *Dunaliella salina* [146].

Fatty-acid profiles are linked to the light cycle [184]. In the diatom *Cyclotella meneghiniana*, total unsaturated fatty acids and the PUFA 20:5ω3 were lowest in the early part of the light period and highest in the dark period. Saturated fatty acids predominated at the beginning of the light period.

The amino-acid composition, in particular free amino acids, varies with growth conditions and growth phase. In the blue-green halophilic alga *Aphonethece halophytica*, the levels of the essential amino acids methionine and phenylalanine are highest at high salinities during log phase [198].

Vitamin levels in algae change with the stage in the growth cycle, light intensity and culture medium. Vitamins are excreted in largest amounts during the stationary phase [21, 153, 183].

Genetic manipulation of algae is an area of expanding interest, and it offers large potential for the development of algae with properties to suit mariculture needs. For example, mutants of *Chlorella vulgaris* [177] and *Chlamydomonas eugametos* [150] with improved vitamin contents have already been prepared.

Other Factors Contributing to Animal Nutrition

Water conditioning by algae

Although the discussion so far has been restricted to the role of nutrients in the algal cell in determining the overall nutrition of feeding animals, growth factors excreted by the algae may also be important. Animals can take up trace ions directly [191] or dissolved organic material, such as vitamins [180], amino acids [140, 188] and simple carbohydrates such as glycollate [52], although such uptake may be negligible in relation to the total carbon requirement of an animal [73]. Such metabolites might have been components of the culture media, compounds actively excreted by healthy algae, or compounds released from dead or decaying material. The beneficial effects of water conditioning by algae has been demonstrated by testing the effects on larval development of filtrates from cultures of different algae. The effects varied from toxic to favourable, depending on the species of microalga and the degree of dilution with normal seawater [224]. For example, *Chlorella* sp. is often added, together with rotifers, to rearing tanks with marine finfish larvae, and whilst the alga serves mainly as food for the rotifers, it also has an undefined beneficial effect on water quality for the fish larvae [106].

Kaolin or silt additives

The addition of kaolin or silt to diets of bivalve molluscs stimulates growth. Kiorboe *et al.* [123] found adding natural silt (10 µm particle size with carbon content of 5%) to an algal diet for *Mytilus edulis* increased the filtration rate and growth, and the organic material of the silt contributed up to 30% of the assimilated material of the algal/silt diet. The addition of kaolin to food was beneficial to the growth of *Crassostrea virginica* juveniles. The kaolin might have enhanced growth

by becoming coated with dissolved nutrients, which would make the nutrients more readily available to the animals, or it may have helped in the physical breakdown and digestion of food particles, or both [130].

Bacteria

Bacteria also play an important role in the growth and development of animals in mariculture systems. Bacteria are present in all mariculture systems; they may be derived from the algal mass culture or the culture tanks in which the animals are maintained, or be indigenous in the seawater. Although axenic culture techniques for rearing *Crassostrea gigas* larvae have been described [128], it is impractical (and probably undesirable) to rear axenic larval or juvenile animals on a large scale. Attempts to obtain axenic *C. gigas* juveniles by use of antibiotics have been unsuccessful [127].

Bacteria may have positive effects on animal nutrition. They may provide food or nutrients [143, 228]. Bacteria in the animal gut could provide essential micronutrients lacking in the alga or algal-fed zooplankton, or aid the digestion of algal components by breaking down polysaccharides and proteins, thereby liberating nutrients that might otherwise be unavailable.

Certain bacteria can (in mariculture systems) act as pathogens of the animals or the algae on which the animals are feeding. A number of bacterial diseases, as well as diseases associated with fungi and viruses, have been documented [20, 186, 187].
Given that bacterial contamination is inevitable in a mariculture laboratory, efforts should be directed at controlling bacterial numbers and pathogenic types to a level within the animal's range of tolerance.

Microencapsulated Artificial Diets

Microencapsulated nutrients and microparticulate artificial feeds have been tested as partial or complete replacements for live algal or zooplankton diets for maricultured animals [30, 75, 104, 105, 125, 129, 200]. The rationale is that the nutritional composition can be controlled and food costs and maintenance might be reduced.

Microcapsules may be prepared in a number of different forms. Either gelatin/acacia-coated capsules or spherical gels of carboxymethyl cellulose are used to deliver water-soluble nutrients of high molecular-weight. Lipid-walled microcapsules deliver water-soluble nutrients of low molecular weight. Lipid-soluble nutrients are incorporated into the lipid wall of the microcapsule.

However, major difficulties have limited the widespread use of microencapsulated diets. No completely artificial diet presently available will support growth as well as does a high-quality live (algae or zooplankton) diet. Chu *et al.* [30] reported the first successful metamorphosis of oyster larvae (*Crassostrea virginica*) on an artificial diet, although the formula included lipid extracted from algae. Feeding trials with juvenile oysters and clams have shown that partial replacement of *Chaetoceros calcitrans* with an artificial diet did not significantly reduce the animals' growth rate [125]. Prawn larvae on an artificial diet successfully grew through to the post-larval stage [104], but their survival rate was lower and more variable, which suggests the diet was not a complete substitute for live algae, or *Artemia* raised on algae.

The microcapsules themselves have problems, including leaching of nutrients, which results in high bacterial numbers and clumping and settling of the food particles.

New methods of preparing lipid-walled capsules that include ethyl cellulose and stearic acid have improved the stability of the capsule wall and reduced leaching [30]. Jones *et al.* [103] coat the capsule with the dietary protein, which is internally cross-linked by chemical treatment. No binders and fillers are needed, the nutrients can all be held within the one capsule, and the microcapsules are very resilient and can withstand dehydration and rehydration without rupture.

Control of bacterial numbers remains a serious limitation in the use of artificial diets. One problem is the difficulty of assessing results of nutritional experiments with microcapsules, since bacteria can compete for the dissolved organic material.

Microcapsules often stay in the upper layers of the water column, and although occasional agitation helps to maintain them in suspension, prolonged agitation may lead to aggregation and sinking, which vastly reduces their availability.

5 Summary

- Microalgae used in mariculture provide essential nutrients for animal growth and development. Algae vary widely in their biochemical composition, which is probably why some algae are better food than others.

- Although some biochemical fractions are more important than others, a deficiency of any of the essential components will reduce the total nutritional value of the alga. Mixed algal diets are likely to be more balanced and therefore better support growth.

- The levels of the polyunsaturated fatty acids, in particular 20:5ω3 and 22:6ω3, show marked variation in algae and are a major factor determining their nutritional value. Most maricultured animals have an essential requirement for 20:5ω3 and 22:6ω3 and even those that do not (as they can synthesise 20:5ω3 and 22:6ω3 from 18:3ω3) show superior growth when these PUFAs are included in their diet. Some animals (e.g. the oyster *C. gigas*) need either 20:5ω3 or 22:6ω3. Prawn larvae (*P. japonicus*) grow better with both PUFAs in the diet. For some animals, the PUFAs 18:3ω3 or the ω6 (e.g. 18:2ω6) may be crucial for proper growth and development.

- The vitamin content of algae varies markedly, and both composition and content are important. Animals may receive some of their requirement by direct uptake from seawater, or from vitamins released by gut microflora (e.g. bacteria).

- Differences in the nutritional value of algae may also be partly a function of differences in the type and content of carbohydrates and the proportions of essential amino acids.

- Other essential nutrients that contribute to the overall nutritive value of an alga are minerals, sterols, hydrocarbons, alkenones and phospholipids.

- Future studies should attempt to gain a better understanding of the nutritional requirements of the animals and of algal biochemistry. This would encourage development of better algal diets through manipulating algal culture conditions, harvesting at different growth stages, developing new clonal isolates and genetic engineering.

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CSIRO Marine Laboratories
comprise

Division of Oceanography
Division of Fisheries

Headquarters
Castray Esplanade, Hobart, Tasmania
GPO Box 1538, Hobart, Tas 7001, Australia

Queensland Laboratory
133 Middle Street, Cleveland, Qld
PO Box 120, Cleveland, Qld 4163

Western Australian Laboratory
Leach Street, Marmion, WA
PO Box 20, North Beach, WA 6020

IBSN 0 643 04833 2