ISSN 0429-9345

Manual on the production and use of live food for aquaculture

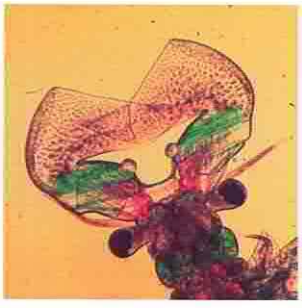
FAO
FISHERIES
TECHNICAL
PAPER

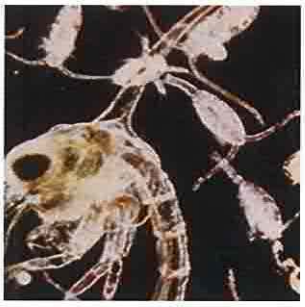
361











Food
and
Agriculture
Organization
of
the
United
Nations

Manual on the production and use of live food for aquaculture

FAO
FISHERIES
TECHNICAL
PAPER

361

Edited by
Patrick Lavens and Patrick Sorgeloos
Laboratory of Aquaculture and Artemia Reference Center
University of Ghent
Ghent, Belgium

Food
and
Agriculture
Organization
of
the
United
Nations

Rome, 1996

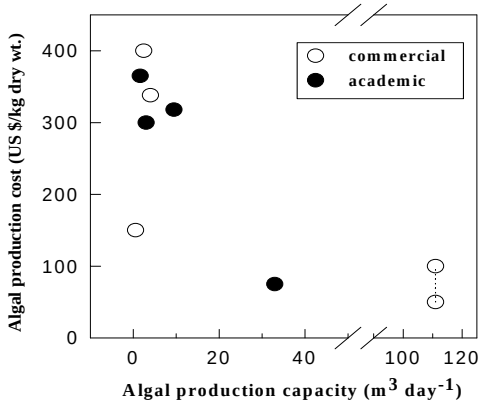


Figure 2.13. Algal production cost as a function of the production capacity for 8 bivalve hatcheries. Filled and unfilled symbols represent data obtained from academic and commercial hatcheries, respectively. The dotted line connects the estimates from one company (modified from Coutteau and Sorgeloos, 1992).

2.4. Nutritional value of micro-algae

The nutritional value of any algal species for a particular organism depends on its cell size, digestibility, production of toxic compounds, and biochemical composition. The gross composition of 16 species of micro-algae is compared in Table 2.12. Although there are marked differences in the compositions of the micro-algal classes and species, protein is always the major organic constituent, followed usually by lipid and then by carbohydrate. Expressed as percentage of dry weight, the range for the level of protein, lipid, and carbohydrate are 12-35%, 7.2-23%, and 4.6-23%, respectively.

The content of highly unsaturated fatty acids (HUFA), in particular eicosapentaenoic acid (20:5n-3, EPA), arachidonic acid (20:4n-6, ARA), and docosahexaenoic acid (22:6n-3, DHA), is of major importance in the evaluation of the nutritional composition of an algal species to be used as food for marine organisms. The fatty acid composition of 10 species of micro-algae grown under defined conditions and harvested during the log phase is presented in Fig. 2.14. Significant concentrations of EPA are present in the diatom species (*Chaetoceros calcitrans*, *C. gracilis*, *S. costatum*, *T. pseudonana*) and the prymnesiophyte *Platymonas lutheri*, whereas high concentrations of DHA are found in the prymnesiophytes (*P. lutheri*, *Isochrysis* sp.) and *Chroomonas salina*.

Micro-algae can also be considered as a rich source of ascorbic acid (0.11-1.62% of dry weight, Fig. 2.15.).

The nutritional value of micro-algae can vary considerably according to the culture conditions. For example the effect of the composition of the culture medium on the proximate composition of various species of micro-algae is demonstrated in Table 2.13.

Table 2.12. Concentrations of chlorophyl a, protein, carbohydrate and lipid in 16 species of micro-algae commonly used in aquaculture (modified from Brown, 1991).

Algal class Species	Dry weight (pg.cell^{-1})	Chl a	Protein	Carbo-hydrate	Lipid
		Weight of constituent (pg.cell^{-1})			
Bacillariophyceae					
Chaetoceros calcitrans	11.3	0.34	3.8	0.68	1.8
Chaetoceros gracilis	74.8	0.78	9.0	2.0	5.2
Nitzchia closterium	-	-	-	-	-
Phaeodactylum tricornutum	76.7	0.41	23.0	6.4	10.7
Skeletonema costatum	52.2	0.63	13.1	2.4	5.0
Thalassiosira pseudonana	28.4	0.27	9.7	2.5	5.5
Chlorophyceae					
Dunaliella tertiolecta	99.9	1.73	20.0	12.2	15.0
Nannochloris atomus	21.4	0.080	6.4	5.0	4.5
Cryptophyceae					
Chroomonas salina	122.5	0.98	35.5	11.0	14.5
Eustigmatophyceae					
Nannochloropsis oculata	6.1	0.054	2.1	0.48	1.1
Prasinophyceae					
Tetraselmis chui	269.0	3.83	83.4	32.5	45.7
Tetraselmis suecica	168.2	1.63	52.1	20.2	16.8
Prymnesiophyceae					
Isochrysis galbana	30.5	0.30	8.8	3.9	7.0
Isochrysis aff. *Galbana* (T-iso)	29.7	0.29	6.8	1.8	5.9
Pavlova lutheri	102.3	0.86	29.7	9.1	12.3
Pavlova salina	93.1	0.34	24.2	6.9	11.2
		Percentage of dry weight			
Bacillariophyceae					
Chaetoceros calcitrans	11.3	3.01	34	6.0	16
Chaetoceros gracilis	74.8	1.04	12	4.7	7.2
Nitzchia closterium	-	-	26	9.8	13
Phaeodactylumtricornutum	76.7	0.53	30	8.4	14
	52.2	1.21	25	4.6	10
Skeletonema costatum					
Thalassiosira pseudonana	28.4	0.95	34	8.8	19
Chlorophyceae					
Dunaliella tertiolecta	99.9	1.73	20	12.2	15
Nannochloris atomus	21.4	0.37	30	23.0	21
Cryptophyceae					
Chroomonas salina	122.5	0.80	29	9.1	12
Eustigmatophyceae					
Nannochloropsis oculata	6.1	0.89	35	7.8	18

Table 2.12.(contd.) Concentrations of chlorophyl a, protein, carbohydrate and lipid in 16 species of micro-algae commonly used in aquaculture (modified from Brown, 1991).

Algal class Species	Dry weight ($pg.cell^{-1}$)	Chl a	Protein	Carbo-hydrate	Lipid
Prasinophyceae					
Tetraselmis chui	269.0	1.42	31	12.1	17
Tetraselmis suecica	168.2	0.97	31	12.0	10
Prymnesiophyceae					
Isochrysis galbana	30.5	0.98	29	12.9	23
Isochrysis aff. *Galbana* (T-iso)	29.7	0.98	23	6.0	20
Pavlova lutheri	102.3	0.84	29	9.0	12
Pavlova salina	93.1	0.98	26	7.4	12

The protein content per cell, which is considered as one of the most important factors determining the nutritional value of micro-algae as feed in aquaculture, was found to be more susceptible to medium-induced variation than the other cellular constituents.

Moreoever, the growth of animals fed a mixture of several algal species is often superior to that obtained when feeding only one algal species. A particular alga may lack a nutrient, while another alga may contain that nutrient and lack a different one. In this way, a mixture of both algal species supplies the animals with an adequate amount of both nutrients. An extensive review of the nutritional aspects of micro-algae used in mariculture of bivalve molluscs, crustaceans, and fish is presented in Brown *et al.* (1989).

Table 2.13. Cellular density (10^6 cells.ml^{-1}) and proximate composition (pg.cell^{-1}) of four marine micro-algae grown in different culture media (Algal-1 is a commercial nutrient) (modified from Herrero *et al.*, 1991)

	Cellular density	Protein	Carbohydrates	Lipids
T. suecica				
Walne	2.29	13.31	6.20	7.04
ES	2.58	16.98	6.93	7.22
F/2	2.38	21.75	8.37	7.92
Algal-1	4.11	32.22	8.83	8.65
D. tertiolecta				
Walne	4.04	13.37	13.22	22.28
ES	4.24	14.88	15.73	23.94
F/2	4.97	13.26	17.91	23.67
Algal-1	8.45	18.82	11.08	18.18
I. galbana				
Walne	10.11	5.17	4.28	25.95
ES	12.09	7.23	5.21	28.38
F/2	10.81	8.13	5.59	26.82
Algal-1	16.15	9.57	4.28	20.68
P. tricornutum				
Walne	19.01	2.65	6.42	6.51
ES	16.23	5.21	9.20	6.45
F/2	24.65	3.34	6.90	5.52
Algal-1	39.04	4.20	5.98	5.79

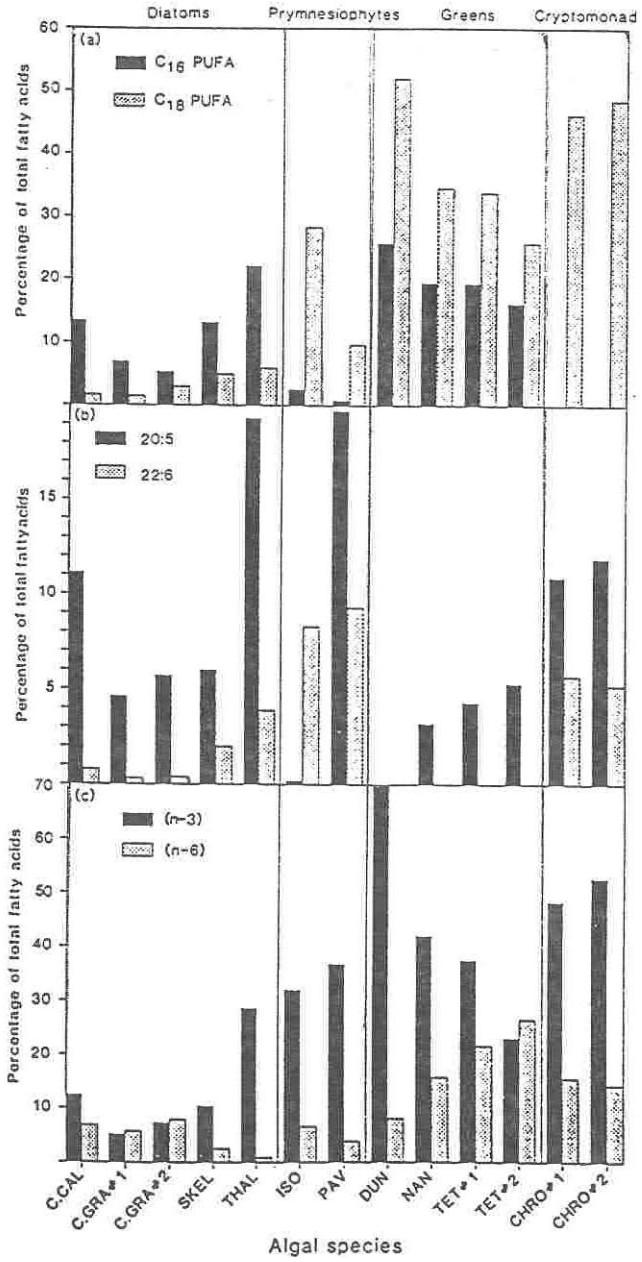


Figure 2.14. Fatty acid composition of 10 species of micro-algae. Relative amounts of (a) C16- and C18-polyunsaturated fatty acids (PUFA); (b) 20:5n-3 and 22:6n-3; (c) (n-3) and (n-6) PUFA. Species abbreviations are: C. CAL: *Chaetoceros calcitrans*; C.GRA: *C. gracilis;* SKEL: *Skeletonema costatum;* THAL: *Thalassiosira pseudonana*; ISO: *Isochrysis sp.* (Tahitian); PAV: *Pavlova lutheri*; DUN: *Dunaliella tertiolecta*; NAN: *Nannochloris atomus*; TET: *Tetraselmis suecica*; CHRO: *Chroomonas salina* (Volkman *et al*., 1989).

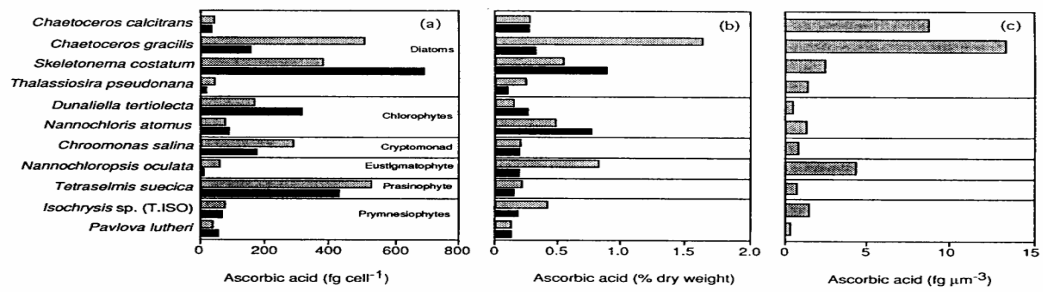


Figure 2.15. Ascorbic acid in microalgae harvested from logarithmic (grey filling) and stationary phase (black filling) cultures, expressed as (a) cellular levels (fg.cell^{-1}), (b) % dry weight, (c) concentrations (fg. µm^{-3}) (Brown and Miller, 1992).

2.5. Use of micro-algae in aquaculture

Micro-algae are an essential food source in the rearing of all stages of marine bivalve molluscs (clams, oysters, scallops), the larval stages of some marine gastropods (abalone, conch), larvae of several marine fish species and penaeid shrimp, and zooplankton.

2.5.1. Bivalve molluscs

Intensive rearing of bivalves has so far relied on the production of live algae, which comprises on average 30% of the operating costs in a bivalve hatchery. The relative algal requirements of the various stages of the bivalve culture process depend on whether the operation aims at the mass-production of larvae for remote setting or growing millions of seed till planting size. In either case, the juveniles, representing the largest biomass in the hatchery and demanding the highest weight-specific rations, consume the largest volumes of algal culture (Fig. 2.16.). The algal species that were reported in an international survey among hatchery operators in 1991 are listed in Table 2.14. Eight algal species (*Isochrysis* sp., clone T-Iso; *C. gracilis*; *C. calcitrans*; *T. suecica*; *T. pseudonana*, clone 3H; *P. lutheri*; *I. galbana*; *S. costatum*) were widely used and represented over 90% of the volume of algal culture produced in 23 facilities.

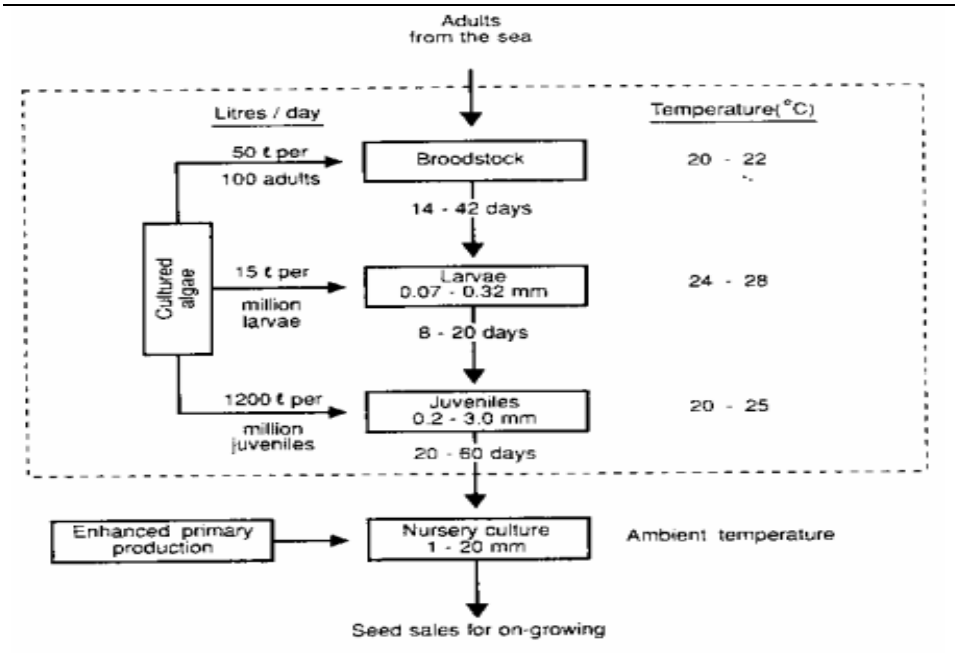


Figure 2.16. Requirements for cultured algae in hatchery and nursery culture of bivalve molluscs (Utting and Spencer, 1991).

Table 2.14. Algal species used in hatchery and nursery rearing of bivalve molluscs as reported in an international questionnaire. Species are ranked according to decreasing frequency of use (Coutteau and Sorgeloos, 1992).

Algal species	frequency of use[†]	total daily production n[‡]	volume (m^3)
Isochrysis sp., clone T-Iso	31	18	23.8
Chaetoceros gracilis	23	11	14.1
Chaetoceros calcitrans	16	10	6.0
Tetraselmis suecica	*15*	*10*	*39.1*
Thalassiosira pseudonana, clone 3H	14	9	112.0
Pavlova lutheri	11	7	11.7
Isochrysis galbana	8	5	9.1
Skeletonema costatum	6	3	58.8
Chroomonas salina	5	3	0.76
Dunaliella tertiolecta	4	2	2.2

Table 2.14. (contd.) Algal species used in hatchery and nursery rearing of bivalve molluscs as reported in an international questionnaire. Species are ranked according to decreasing frequency of use (Coutteau and Sorgeloos, 1992)

Chaetoceros simplex	3	3	1.76
Chaetoceros muelleri	3	2	5.0
Nannochloropsis sp.	3	2	0.20
Cyclotella sp.	2	1	0.36
Phaeodactylum tricornutum	2	1	2.0
Tetraselmis chui	2	0	-
Pavlova salina	1	1	3.18
Dicruteria sp.	1	1	4.07
Tetraselmis levis	1	0	-
Dunaliella perva	1	1	0.012
Thalassiosira weissfloggii	1	1	0.12
Chlamydomonas sp.	1	1	0.52
Chlorella sp.	1	1	0.36
TOTAL	43	23	295

†: number of hatcheries growing each algal species (from 43 completed forms)
‡: number of hatcheries providing data which allowed to calculate daily production per algal species (from 23 completed foms)

The larvae of most bivalve species have similar food preferences; suitable algal species including *C. calcitrans*, *T. pseudonana* (3H), *I. galbana*, and *T. suecica* (for larvae > 120 µm in length). Combinations of flagellates and diatoms provide a well balanced diet which will generally accelerate the rate of larval development to metamorphosis in comparison with unialgal diets. The quantity fed depends upon the larval density, but suitable cell concentrations (expressed as cells.µl^{-1}) are given by each of the following combinations:

- *I. galbana*; 50
- *C. calcitrans*; 250
- *I. galbana*/*C. calcitrans*; 25/125
- *I. galbana*/*C. calcitrans*/*T. suecica*; 33/83/3.3 (larvae > 120µm)

Because of the high cost of cultured algae, bivalve hatcheries prefer to move juveniles to outdoor nursery systems at a maximum size of 1-2 mm length. In this way, the duration of the juvenile phase in closely controlled hatchery conditions is relatively short for oysters at about 20 days but much longer for the slower growing clams at up to 60 days. Bivalve food rations are preferentially expressed as daily weight-specific rations, such as number of cells or percent dry weight of algae per live weight of bivalves. Seed growth is largely influenced by food ration and the optimal ration for maximum growth depends upon the species and culture conditions of the algae making up the diet, and the bivalve culture conditions. Under practical hatchery conditions, high food rations are often fed, which may be as high as 5-6% dry weight of algae per live weight of spat per day.

2.5.2. Penaeid shrimp

A typical algal feeding regime for penaeid larvae is given in Table 2.15. Algae are added during the non-feeding nauplius stage so that algae are available immediately upon molting into the protozoea stage. Algal species most often used are *Tetraselmis chui*, *Chaetoceros gracilis*, and *Skeletonema costatum*. As feeding preference changes from primarily herbivorous to carnivorous during the mysis stages, the quantity of algae is reduced. Nevertheless, a background level of algae is maintained as this may stabilize water quality. The "same-tank method", in which the algae are cultured in the same water as that of the larvae using sunlight and fertilizers, was originally developed in Japan for culturing larval *Penaeus japonicus* and is extensively described by Liao *et al.* (1993).

Table 2.15: Typical algal feeding regimes (cells.ml^{-1}) for penaeid larvae (N: nauplius, P: protozoea, M: mysis, PL: postlarva stage) (modified from Smith *et al.*, 1993b).

Substage	*Chaetoceros neogracile (C. gracilis)*	*Tetraselmis chuii*
N_5 or N_6	60,000	0-15,000
P_1	100,000-120,000	30,000
P_2	120,000	35,000
P_3	120,000	35,000
M_1	100,000	30,000
M_2	75,000	20,000
M_3	50,000-75,000	20,000
PL_1 to PL_5	20,000-75,000	5,000-20,000

2.5.3. Marine fish

Apart from the requirement for micro-algae for culturing and/or enriching live prey organisms such as *Artemia* and rotifers (see Chapters 3. and 4.3.), algae are often used directly in the tanks for rearing marine fish larvae. This "green water technique" is part of the commonly applied techniques for rearing larvae of gilthead seabream *Sparus aurata* (50,000 cells ml^{-1} of *Isochrysis* sp. + 400,000 cells.ml^{-1} of *Chlorella* sp. per day), milkfish *Chanos chanos* (between 500 and 3,500 *Chlorella* cells.ml^{-1} are added from hatching till day 21), Mahimahi *Coryphaena hippurus* (200,000 cells.ml^{-1} of either *Chaetoceros gracilis*, *Tetraselmis chui*, or

Chlorella sp.), halibut *Hippoglossus hippoglossus* (*Tetraselmis* sp.), and turbot *Scophthalmus maximus* (60,000 cells.ml^{-1} of *Tetraselmis* sp. or 130,000 cells.ml^{-1} of *I. galbana*).

The effects of the presence of micro-algae in the larval rearing tank are still not fully understood and include:

- stabilizing the water quality in static rearing systems (remove metabolic by-products, produce oxygen);
- a direct food source through active uptake by the larvae with the polysaccharides present in the algal cell walls possibly stimulating the non-specific immune system in the larvae;
- an indirect source of nutrients for fish larvae through the live feed (i.e. by maintaining the nutritional value of the live prey organisms in the tank);
- increasing feeding incidence by enhancing visual contrast and light dispersion, and
- microbial control by algal exudates in tank water and/or larval gut.

2.6. Replacement diets for live algae

The high costs associated with algal production, the risks for contamination, and temporal variations in the algal food value still pose problems for any aquaculture operation depending on the mass-cultures of unicellular algae. In order to overcome or reduce the problems and limitations associated with algal cultures, various investigators have attempted to replace algae by using artificial diets either as a supplement or as the main food source. Different approaches are being applied to reduce the need for on-site algal production, including the use of preserved algae, micro-encapsulated diets, and yeast-based feeds.

To date, the requirement for live algae in the mass-production of prey-organisms has been largely reduced. In this way, baker's yeast, marine yeasts and lipid-enriched yeast diets are now routinely used as a sole diet or in combination with the alga *Chlorella* for rearing the rotifer *B. plicatilis* (see Chapter 3). In addition, considerable progress has been made in the replacement of live algae in the larval rearing of commercially important shrimp species. Partial replacement of live algae using micro-encapsulated and yeast-based diets is now routine in hatcheries for penaeid shrimp. Complete substitution of live algae by a commercial micro-encapsulated diet has been accomplished recently for the production of various penaeid species using seawater filtered to 5 µm, eliminating the algae but not the bacteria, which apparently contribute important micronutrients (and possibly immunostimulants). In marine fish hatcheries, the tendency is to apply a "clear water technique" instead of a "green water technique". However, the omission of algae in the larval tanks, which requires optimization of feeding strategies and zootechnical aspects, still often results in less predictable culture performance. Despite extensive research efforts, the use of artificial diets in the culture of bivalve molluscs is still very limited. The advantages and disadvantages of each of the three classes of replacement diets for live algae are briefly discussed below.

2.6.1. Preserved algae

A possible alternative to on-site algal culture could be the distribution of preserved algae that are produced at relatively low cost in a large facility under optimal climatological conditions and using the most cost-effective production systems. Centrifugation of algae into a paste form and subsequent refrigeration until required is widely applied in North America by oyster hatcheries using remote setting techniques. However, the limited shelf-life and/or the high prices of the presently available algal pastes (US$ 200 or more per kg dry weight) have discouraged many growers from using them. Recently, the development of preservation techniques has extended the shelf-life of *Thalassiosira pseudonana* concentrates from about 10 days to more than one year, which makes it possible to utilize excess and off-season algal production. Outdoor pond production on a large scale has lead to the bulk availability of a limited number of "algal meals", such as spray-dried *Spirulina* and a spray-dried extract of *Dunaliella salina*. The latter may be used as a supplement to live algae to improve the growth of bivalve larvae.

In addition, recent techniques have been developed for the large scale production of marine micro-algae under heterotrophic growth conditions, by utilizing organic carbon instead of light as an energy source. Heterotrophic algal cultures can attain up to 1,000 times higher densities than photoautotrophic cultures and can be preserved by spray-drying. Projected costs of producing algae within industrial fermentors vary from US$ 5 to 25 per kg (Gladue, 1991). Unfortunately, heterotrophic mass-production techniques have only been realized for a few algal species, and most of the species that are known to be of high nutritional value (*e.g. Chaetoceros*, *Isochrysis*, *Skeletonema*, *Thalassiosira*, *Monochrysis*) are not capable of growing in the dark. Furthermore, heterotrophic conditions may result in a drastic change in the gross composition and reduced (n-3) HUFA content as compared to light-grown algae. Nevertheless, further developments in this rather new technology may improve the biochemical composition and the range of dried algae available in the future.

2.6.2. Micro-encapsulated diets

Through micro-encapsulation techniques dietary ingredients can be encapsulated within digestible capsules and delivered to suspension-feeders without losses of nutrients to the aqueous medium. Possible problems arising from the use of microparticulate feeds include settling, clumping and bacterial degradation of the particles, leaching of nutrients, and low digestibility of the cell wall material. In this regard, low susceptibility to bacterial attack and high digestibility for the filter-feeder may be conflicting requirements for a capsule wall.

2.6.3. Yeast-based diets

Because of their suitable particle size and high stability in the water column yeasts can easily be removed from suspension and ingested by filter-feeding organisms. Furthermore, as opposed to most of the other alternatives to live algae, yeasts can be mass-produced at a relatively low cost. The potential of yeasts as a food in aquaculture has been proven by their successful application in the rearing of rotifers and some species of penaeid shrimp. However, a limited nutritional value of yeasts was reported for various species of filter-feeders and attributed to their nutritionally deficient composition and/or undigestible cell wall. Despite this, the nutritional value and digestibility of yeast-based diets can be improved through the addition of limiting essential nutrients and the chemical treatment of the yeast

cell wall, respectively. In this way, about 50% of the algae can be substituted by yeast-based diets with minimal effects on the growth of juvenile hard clam, *Mercenaria mercenaria* (Coutteau *et al.*, 1994).

2.7. Literature of interest

Anonymous, 1991. The design and operation of live feeds production systems. In: Rotifer and micro-algae culture systems, Fulks, W. and Main K.L. (Eds.). Proceedings of a US-Asia Workshop, Honolulu, Hawaii, January 28-31, 1991The Oceanic Institute, Hawaii, USA, pp 3-52.

Barnabé, G. 1990. Harvesting micro-algae. In: Aquaculture, Volume I. Barnabé, G. (Ed.). Ellis Horwood, New York, pp 207-212.

Brown, M.R., Jeffrey, S.W. and Garland, C.D. 1989. Nutritional aspects of microalgae used in mariculture; a literature review, CSIRO Marine Laboratories Report 205, 44 pp.

Brown, M.R. 1991. The amino acid and sugar composition of 16 species of microalgae used in mariculture. Aquaculture, 145: 79-99.

Brown, M.R. and Miller, K.A. 1992. The ascorbic acid content of eleven species of microalgae used in mariculture. J. Appl. Phycol., 4: 205-215.

Coutteau, P. and Sorgeloos, P. 1992. The requirement for live algae and their replacement by artificial diets in the hatchery and nursery rearing of bivalve molluscs: an international survey. J. Shellfish Res., 11(2): 467-476.

Coutteau, P., Hadley, N., Manzi, J. and Sorgeloos, P. 1994. Effect of algal ration and substitution of algae by manipulated yeast diets on growth of juvenile *Mercenaria mercenaria*. Aquaculture, 120: 135-150.

De Pauw, N. and Persoone, G. 1988. Micro-algae for aquaculture. In: Micro-algal Biotechnology. Borowitzka, M.A. and L.J. Borowitzka (Eds.). Cambridge University Press, Cambridge, U.K., pp 197-221.

Donaldson, J. 1991. Commercial production of microalgae at Coast Oyster Company. In: Rotifer and microalgae culture systems, Proceedings of a US-Asia Workshop, Honolulu, Hawaii, January 28-31, 1991. Fulks, W. and K.L. Main (eds). The Oceanic Institute, Hawaii, USA, pp 229-236.

Fox, J.M. 1983. Intensive algal culture techniques. In: CRC Handbook of mariculture. Volume 1. Crustacean Aquaculture. McVey J P (Ed.). CRC Press, Inc., Boca Raton, Florida, USA, pp 43-69.

Gladue, R. 1991. Heterotrophic microalgae production: Potential for application to aquaculture feeds. In: Rotifer and microalgae culture systems, Proceedings of a US-Asia Workshop, Honolulu, Hawaii, January 28-31, 1991. Fulks, W and K.L. Main (eds). The Oceanic Institute, Hawaii, USA, pp 276-286.

Helm, M.M., Laing I., and Jones, E. 1979. Culture of algae for larval fish and shellfish rearing. Part 1. The development of a 200 l algal culture vessel at Conwy, Fisheries Research Technical Report, MAFF Direct. Fish. Res., Lowestoft (53), UK, 18 pp.

Herrero, C., C. Angeles, J. Fabregas, and J. Abalde 1991. Yields in biomass and chemical constituents of four commercially important marine microalgae with different culture media. Aquacult. Eng., 10: 99-110.

Laing, I. 1991. Cultivation of marine unicellular algae. MAFF Laboratory Leaflet Number 67. Directorate of Fisheries Research Lowestoft, UK. 31pp.

Lee, C-S and Tamaru, C.S. 1993. Live larval food production at the Oceanic Institute, Hawai. In: CRC Handbook of mariculture. Volume 1. Crustacean Aquaculture, 2nd Edition. McVey J.P. (Ed.). CRC Press, Inc., Boca Raton, Florida, USA, pp 15-28.

Liao, I., Su, H.-S. and Lin, J.-H 1993. Larval foods for penaeid prawns. In: CRC Handbook of mariculture. Volume 1. Crustacean Aquaculture, 2nd Edition. McVey J.P. (Ed.). CRC Press, Inc., Boca Raton, Florida, USA, pp 29-59.

Palanisamy, V., Latif, F.A. and Resat, R.B.M. 1991. A guide on the production of algal culture for use in shrimp hatcheries. National Prawn Fry Production and Research Centre, Pulau Sayak, Kedah, Department of Fisheries, Ministry of Agriculture, Malaysia. 23pp.

Sheldon, R.W. and Parsons T.R. 1967. A practical manual on the use of the Coulter counter in marine research. Coulter Electronics Sales Company, Ontario, Canada, 65 pp.

Smith, L.L., Fox, J.M. and Granvil, D.R. 1993a. Intensive algae culture techniques. In: CRC Handbook of mariculture. Volume 1. Crustacean Aquaculture, 2nd Edition. McVey J.P. (Ed.). CRC Press, Inc., Boca Raton, Florida, USA, pp 3-13.

Smith, L.L., Fox, J.M. and Granvil, D.R. 1993b. Intensive larviculture techniques. In: CRC Handbook of mariculture. Volume 1. Crustacean Aquaculture, 2nd Edition. McVey J.P. (Ed.). CRC Press, Inc., Boca Raton, Florida, USA, pp 153-172.

Utting, S.D. and Spencer, B.E. 1991. The hatchery culture of bivalve mollusc larvae and juveniles. MAFF Laboratory Leaflet Number 68. Directorate of Fisheries Research, Lowestoft, UK. 31pp.

Volkman, J.K., Jeffrey, S.W., Nichols, P.D, Rogers, G.I. and Garland, C.D. 1989. Fatty acids and lipid classes of ten species of microalgae used in mariculture. J. Exp. Mar. Biol. Ecol., 128: 219-240.

Vonshak, A. 1986. Laboratory techniques for the cultivation of microalgae. In: CRC Handbook of microalgal mass culture. Richmond A. (Ed.). CRC Press, Inc., Boca Raton, Florida, USA, pp 117-145.

Walsh, D.T., Withstandley, C.A., Kraus, R.A., and Petrovits, B.J. 1987. Mass culture of selected marine microalgae for the nursery production of bivalve seed. J. Shellfish Res., 6: 71-77.