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Manual on the production and use of live food for aquaculture

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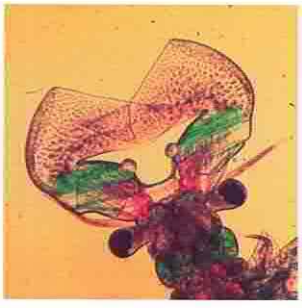

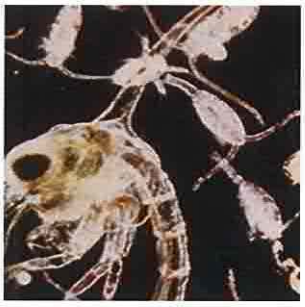

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2. MICRO-ALGAE

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2.1. Introduction

Phytoplankton comprises the base of the food chain in the marine environment. Therefore, micro-algae are indispensable in the commercial rearing of various species of marine animals as a food source for all growth stages of bivalve molluscs, larval stages of some crustacean species, and very early growth stages of some fish species. Algae are furthermore used to produce mass quantities of zooplankton (rotifers, copepods, brine shrimp) which serve in turn as food for larval and early-juvenile stages of crustaceans and fish (Fig. 2.1.). Besides, for rearing marine fish larvae according to the "green water technique"
algae are used directly in the larval tanks, where they are believed to play a role in stabilizing the water quality, nutrition of the larvae, and microbial control.

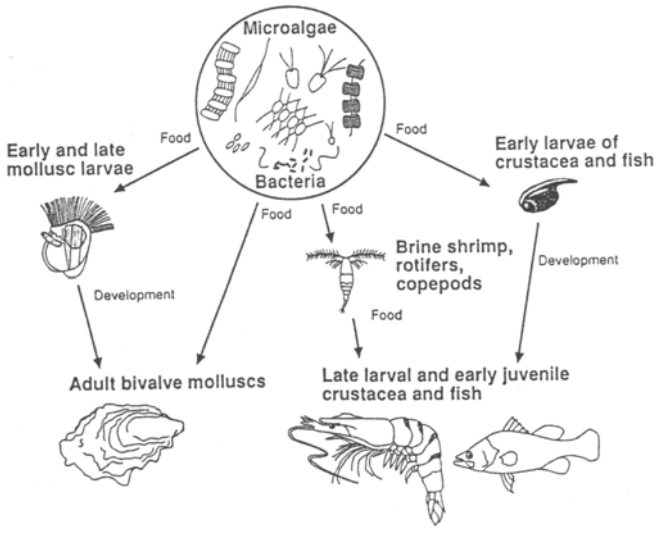

Figure 2.1. The central role of micro-algae in mariculture (Brown *et al*., 1989).

All algal species are not equally successful in supporting the growth and survival of a particular filter-feeding animal. Suitable algal species have been selected on the basis of their mass-culture potential, cell size, digestibility, and overall food value for the feeding animal. Various techniques have been developed to grow these food species on a large scale, ranging from less controlled extensive to monospecific intensive cultures. However, the controlled production of micro-algae is a complex and expensive procedure. A possible alternative to on-site algal culture is the collection of algae from the natural environment where, under certain conditions, they may be extremely abundant. Furthermore, in order to overcome or reduce the problems and limitations associated with algal cultures, various

investigators have attempted to replace algae using artificial diets either as a supplement or as the main food source. These various aspects of the production, use and substitution of micro-algae in aquaculture will be treated within the limits of this chapter.

2.2. Major classes and genera of cultured algal species

Today, more than 40 different species of micro-algae, isolated in different parts of the world, are cultured as pure strains in intensive systems. Table 2.1. lists the eight major classes and 32 genera of cultured algae currently used to feed different groups of commercially important aquatic organisms. The list includes species of diatoms, flagellated and chlorococcalean green algae, and filamentous blue-green algae, ranging in size from a few micrometer to more than 100 µm. The most frequently used species in commercial mariculture operations are the diatoms *Skeletonema costatum*, *Thalassiosira pseudonana*, *Chaetoceros gracilis*, *C. calcitrans*, the flagellates *Isochrysis galbana*, *Tetraselmis suecica*, *Monochrysis lutheri* and the chlorococcalean *Chlorella* spp. (Fig. 2.2.).

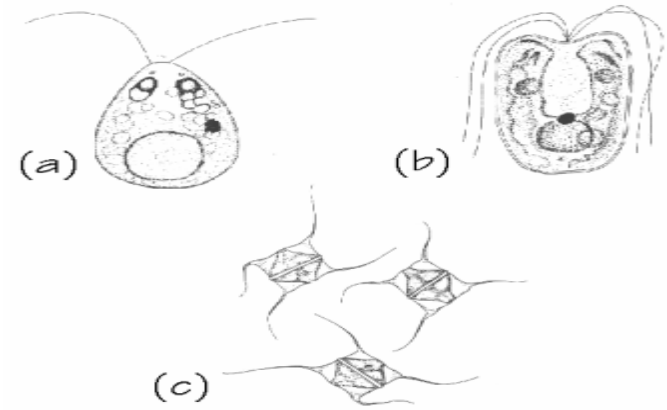

Figure 2.2. Some types of marine algae used as food in aquaculture (a) *Tetraselmis* spp. (b) *Dunaliella* spp. (c) *Chaetoceros* spp. (Laing, 1991).

Table 2.1. Major classes and genera of micro-algae cultured in aquaculture (modified from De Pauw and Persoone, 1988) .

Class	Genus	Examples of application
Bacillariophyceae	*Skeletonema*	PL,BL,BP
	Thalassiosira	PL,BL,BP
	Phaeodactylum	PL,BL,BP,ML,BS
	Chaetoceros	PL,BL,BP,BS
	Cylindrotheca	PL
	Bellerochea	BP
	Actinocyclus	BP
	Nitzchia	BS
	Cyclotella	BS
Haptophyceae	*Isochrysis*	PL,BL,BP,ML,BS
	Pseudoisochrysis	BL,BP,ML
	dicrateria	BP
Chrysophyceae	*Monochrysis (Pavlova)*	BL,BP,BS,MR
Prasinophyceae	*Tetraselmis (Platymonas)*	PL,BL,BP,AL,BS,MR
	Pyramimonas	BL,BP
	Micromonas	BP
Cryptophyceae	*Chroomonas*	BP
	Cryptomonas	BP
	Rhodomonas	BL,BP
Cryptophyceae	*Chlamydomonas*	BL,BP,FZ,MR,BS
	Chlorococcum	BP
Xanthophyceae	*Olisthodiscus*	BP
Chlorophyceae	*Carteria*	BP
	Dunaliella	BP,BS,MR
Cyanophyceae	*Spirulina*	PL,BP,BS,MR

PL, penaeid shrimp larvae; BL, bivalve mollusc larvae; ML, freshwater prawn larvae; BP, bivalve mollusc postlarvae; AL, abalone larvae; MR, marine rotifers (*Brachionus*); BS, brine shrimp (*Artemia*); SC, saltwater copepods; FZ, freshwater zooplankton

2.3. Algal production

2.3.1. Physical and chemical conditions

The most important parameters regulating algal growth are nutrient quantity and quality, light, pH, turbulence, salinity and temperature. The most optimal parameters as well as the tolerated ranges are species specific and a broad generalization for the most important parameters is given in Table 2.2. Also, the various factors may be interdependent and a parameter that is optimal for one set of conditions is not necessarily optimal for another.

2.3.1.1. Culture medium/nutrients

Concentrations of cells in phytoplankton cultures are generally higher than those found in nature. Algal cultures must therefore be enriched with nutrients to make up for the deficiencies in the seawater. Macronutrients include nitrate, phosphate (in an approximate ratio of 6:1), and silicate.

Table 2.2. A generalized set of conditions for culturing micro-algae (modified from Anonymous, 1991).

Parameters	Range	Optima
Temperature (°C)	16-27	18-24
Salinity ($g.l^{-1}$)	12-40	20-24
Light intensity (lux)	1,000-10,000 (depends on volume and density)	2,500-5,000
Photoperiod (light:dark, hours)		16:8 (minimum) 24:0 (maximum)
pH	7-9	8.2-8.7

Silicate is specifically used for the growth of diatoms which utilize this compound for production of an external shell. Micronutrients consist of various trace metals and the vitamins thiamin (B_1), cyanocobalamin (B_{12}) and sometimes biotin. Two enrichment media that have been used extensively and are suitable for the growth of most algae are the Walne medium (Table 2.3.) and the Guillard's $F/_2$ medium (Table 2.4.). Various specific recipes for algal culture media are described by Vonshak (1986). Commercially available nutrient solutions may reduce preparation labour. The complexity and cost of the above culture media often excludes their use for large-scale culture operations. Alternative enrichment media that are suitable for mass production of micro-algae in large-scale extensive systems contain only the most essential nutrients and are composed of agriculture-grade rather than laboratory-grade fertilizers (Table 2.5.).

Table 2.3. Composition and preparation of Walne medium (modified from Laing, 1991).	
Constituents	Quantities
Solution A (at 1 ml per liter of culture)	
Ferric chloride ($FeCl_3$)	0.8 g[(a)]
Manganous chloride ($MnCl_2.4H_2O$)	0.4 g
Boric acid (H_3BO_3)	33.6 g
EDTA[(b)], di-sodium salt	45.0 g
Sodium di-hydrogen orthophosphate ($NaH_2PO_4.2H_2O$)	20.0 g
Sodium nitrate ($NaNO_3$)	100.0 g
Solution B	1.0 ml
Make up to 1 litre with fresh water [(c)]	Heat to dissolve
Solution B	
Zinc chloride ($ZnCl_2$)	2.1 g
Cobaltous chloride ($CoCl_2.6H_2O$)	2.0 g
Ammonium molybdate ($(NH_4)_6Mo_7O_{24}.4H_2O$)	0.9 g
Cupric sulphate ($CuSO_4.5H_2O$)	2.0 g
Concentrated HCl	10.0 ml
Make up to 100 ml fresh water [(c)]	Heat to dissolve
Solution C (at 0.1 ml per liter of culture)	
Vitamin B_1	0.2 g
Solution E	25.0 ml
Make up to 200 ml with fresh water [(c)]	
Solution D (for culture of diatoms-used in addition to solutions A and C, at 2 ml per liter of culture)	
Sodium metasilicate ($Na_2SiO_3.5H_2O$)	40.0 g
Make up to 1 litre with fresh water [(c)]	Shake to dissolve
Solution E	
Vitamin B_{12}	0.1 g
make up to 250 ml with fresh water [(c)]	
Solution F (for culture of *Chroomonas salina* - used in addition to solutions A and C, at 1 ml per liter of culture)	
Sodium nitrate ($NaNO_3$)	200.0 g
make up to 1 litre with fresh water[(c)]	

(a) Use 2.0 g for culture of *Chaetoceros calcitrans* in filtered sea water; (b) Ethylene diamine tetra acetic acid; (c) Use distilled water if possible.

Table 2.4. Composition and preparation of Guillard's F/$_2$ medium (modified from Smith *et al.*, 1993a).

Nutrients	Final concentration (mg.l^{-1} seawater)[a]	Stock solution preparations
$NaNO_3$	75	**Nitrate/Phosphate Solution** Working Stock: add 75 g $NaNO_3$ + 5 g NaH_2PO_4 to 1 liter distilled water (DW)
$NaH_2PO_4.H_2O$	5	
$Na_2SiO_3.9H_2O$	30	**Silicate Solution** Working Stock: add 30 g Na_2SiO_3 to 1 liter DW
$Na_2C_{10}H_{14}O_8N_2.H_2O$ (Na_2EDTA)	4.36	**Trace Metal/EDTA Solution** Primary stocks: make 5 separate 1-liter stocks of (g.l^{-1} DW) 10.0 g $CoCl_2$, 9.8 g $CuSO_4$, 180 g $MnCl_2$, 6.3 g Na_2MoO_4, 22.0 g $ZnSO_4$
$CoCl_2.6H_2O$	0.01	
$CuSO_4.5H_2O$	0.01	
$FeCl_3.6H_2O$	3.15	
$MnCl_2.4H_2O$	0.18	Working stock: add 1 ml of each primary stock solution + 4.35 g $Na_2C_{10}H_{14}O_8N_2$ + 3.15 g $FeCl_3$ to 1 liter DW
$Na_2MoO_4.2H_2O$	0.006	
$ZnSO_4.7H_2O$	0.022	
Thiamine HCl	0.1	**Vitamin Solution** Primary stock: add 20 g thiamin HCl + 0.1 g biotin + 0.1 g B_{12} to 1 liter DW
Biotin	0.0005	
B_{12}	0.0005	Working stock: add 5 ml primary stock to 1 liter DW

Table 2.5. Various combinations of fertilizers that can be used for mass culture of marine algae (modified from Palanisamy *et al.*, 1991).

Fertilizers	Concentration ($mg.l^{-1}$)					
	A	B	C	D	E	F
Ammonium sulfate	150	100	300	100	-	-
Urea	7.5	5	-	10-15	-	12-15
Calcium superphosphate	25	15	50	-	-	-
Clewat 32	-	5	-	-	-	-
N:P 16/20 fertilizer	-	-	-	10-15	-	-
N:P:K 16-20-20	-	-	-	-	12-15	-
N:P:K 14-14-14	-	-	-	-	-	30

2.3.1.2. Light

As with all plants, micro-algae photosynthesize, *i.e.* they assimilate inorganic carbon for conversion into organic matter. Light is the source of energy which drives this reaction and in this regard intensity, spectral quality and photoperiod need to be considered. Light intensity plays an important role, but the requirements vary greatly with the culture depth and the density of the algal culture: at higher depths and cell concentrations the light intensity must be increased to penetrate through the culture (*e.g.* 1,000 lux is suitable for erlenmeyer flasks, 5,000-10,000 is required for larger volumes). Light may be natural or supplied by fluorescent tubes. Too high light intensity (*e.g.* direct sun light, small container close to artificial light) may result in photo-inhibition. Also, overheating due to both natural and artificial illumination should be avoided. Fluorescent tubes emitting either in the blue or the red light spectrum should be preferred as these are the most active portions of the light spectrum for photosynthesis. The duration of artificial illumination should be minimum 18 h of light per day, although cultivated phytoplankton develop normally under constant illumination.

2.3.1.3. pH

The pH range for most cultured algal species is between 7 and 9, with the optimum range being 8.2-8.7. Complete culture collapse due to the disruption of many cellular processes can result from a failure to maintain an acceptable pH. The latter is accomplished by aerating the culture (see below). In the case of high-density algal culture, the addition of carbon dioxide allows to correct for increased pH, which may reach limiting values of up to pH 9 during algal growth.

2.3.1.4. Aeration/mixing

Mixing is necessary to prevent sedimentation of the algae, to ensure that all cells of the population are equally exposed to the light and nutrients, to avoid thermal stratification (*e.g.* in outdoor cultures) and to improve gas exchange between the culture medium and the air. The latter is of primary importance as the air contains the carbon source for photosynthesis in the form of carbon dioxide. For very dense cultures, the CO_2 originating from the air (containing 0.03% CO_2) bubbled through the culture is limiting the algal growth and pure carbon dioxide may be supplemented to the air supply (*e.g.* at a rate of 1% of the volume of air). CO_2 addition furthermore buffers the water against pH changes as a result of the CO_2/HCO_3^- balance. Depending on the scale of the culture system, mixing is achieved by stirring daily by hand (test tubes, erlenmeyers), aerating (bags, tanks), or using paddle wheels and jetpumps (ponds). However, it should be noted that not all algal species can tolerate vigorous mixing.

2.3.1.5. Temperature

The optimal temperature for phytoplankton cultures is generally between 20 and 24°C, although this may vary with the composition of the culture medium, the species and strain cultured. Most commonly cultured species of micro-algae tolerate temperatures between 16 and 27°C. Temperatures lower than 16°C will slow down growth, whereas those higher than 35°C are lethal for a number of species. If necessary, algal cultures can be cooled by a flow of cold water over the surface of the culture vessel or by controlling the air temperature with refrigerated air- conditioning units.

2.3.1.6. Salinity

Marine phytoplankton are extremely tolerant to changes in salinity. Most species grow best at a salinity that is slightly lower than that of their native habitat, which is obtained by diluting sea water with tap water. Salinities of 20-24 $g.l^{-1}$ have been found to be optimal.

2.3.2. Growth dynamics

The growth of an axenic culture of micro-algae is characterized by five phases (Fig. 2.3.):

- lag or induction phase

  This phase, during which little increase in cell density occurs, is relatively long when an algal culture is transferred from a plate to liquid culture. Cultures inoculated with exponentially growing algae have short lag phases, which can seriously reduce the time required for upscaling. The lag in growth is attributed to the physiological adaptation of the cell metabolism to growth, such as the increase of the levels of enzymes and metabolites involved in cell division and carbon fixation.

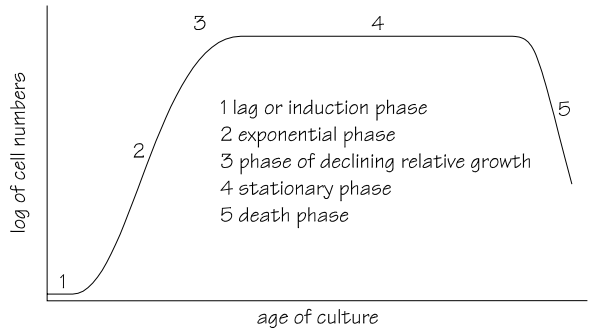

Figure 2.3. Five growth phases of micro-algae cultures.

- exponential phase

During the second phase, the cell density increases as a function of time t according to a logarithmic function:

$C_t = C_0.e^{mt}$

with C_t and C_0 being the cell concentrations at time t and 0, respectively, and m = specific growth rate. The specific growth rate is mainly dependent on algal species, light intensity and temperature.

- phase of declining growth rate

Cell division slows down when nutrients, light, pH, carbon dioxide or other physical and chemical factors begin to limit growth.

- stationary phase

In the fourth stage the limiting factor and the growth rate are balanced, which results in a relatively constant cell density.

- death or "crash" phase

During the final stage, water quality deteriorates and nutrients are depleted to a level incapable of sustaining growth. Cell density decreases rapidly and the culture eventually collapses.

In practice, culture crashes can be caused by a variety of reasons, including the depletion of a nutrient, oxygen deficiency, overheating, pH disturbance, or contamination. The key to the success of algal production is maintaining all cultures in the exponential phase of growth. Moreover, the nutritional value of the produced algae is inferior once the culture is beyond phase 3 due to reduced digestibility, deficient composition, and possible production of toxic metabolites.