Santhanam Perumal
Thirunavukkarasu A.R.
Perumal Pachiappan *Editors*

Advances in Marine and Brackishwater Aquaculture

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Dedicated to the coastal fisher-folks and fish farmers

Foreword

Fisheries play an important role in augmenting nutritional security and employment to millions of rural folk worldwide. The global fish production is in the order of 145 million tonnes more or less equally contributed by capture and culture fisheries showing annual growth rate of 4.7 % and it is one of the fastest growing food sectors. Production through capture fisheries in many areas is showing a declining trend or is stagnating and reaching the maximum sustainable yield (MSY). This has forced to look for aquaculture as a potential alternate for fish production. The demand for fish and fishery products is increasing at a faster phase, and to meet this demand, technologies for producing more fish is the need of the hour. In this context, capture fisheries has limitations since constant catch efforts and increased exploitation have reduced resources, which will be further depleted. It will become difficult to increase or restore and even sustain fishery. Hence, globally all-out efforts are made for aquaculture development through various technologies for growing diversified organisms adopting different practices in various culture systems.

Indian aquaculture is in the growing trend, but the phase of development is slow. Though a phenomenal growth was expected considering the vast potential in line with the developments witnessed in 1980s and 1990s, the development was hampered due to unexpected social and environmental issues coupled with the outbreak of uncontrollable diseases. The sustainability of aquaculture itself has become difficult. One of the reasons attributed was over dependence on a single group of organisms, like shrimp, for farming. This has prompted for diversification to other species farming and systems and practices.

Various research and development institutions are making efforts for developing technologies, transferring the same for field applications. However, the process is very slow and requires concerted efforts of all concerned with cooperation and commitment. This only will pave way for further development of technologies, their dissemination and application. In this context, the book *Advances in Marine and Brackishwater Aquaculture* is one of the meaningful efforts. An attempt has been made to bring out a comprehensive information on marine and brackishwater aquaculture of different organisms compiling the articles of experts in this field.

I am sure that this book would be highly useful to students, research workers, planners, farmers and entrepreneurs involved in aquaculture. I express my warm appreciation to all the authors and organizers for their great contributions in bringing out this book.

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Preface

The marine and brackishwater aquaculture is defined as the rearing of marine or brackishwater organisms under controlled environmental and feed conditions. Aquaculture is a rapidly growing food production industry, accounting for over one-third of global fisheries production and is a lucrative industry in the domestic and international markets. The interest in marine and brackishwater aquaculture has dramatically increased with the recent failures in the capture fisheries. Fishery production supplied the world with about 154 million tonnes of fish in 2010 (FAO 2012). Of this, over 115 million tonnes was used as human food, providing an estimated apparent per capita supply of about 17 kg (live weight equivalent). Recently, Aquaculture production accounted for 46 % of total food fish supply, a slightly lower proportion than reported in 2008, because of major downward trend in aquaculture and capture fishery production. The per capita supply has remained fairly static in recent years as growth in supply from aquaculture has offset a small decline in capture fishery production and a rising population. In 2008, per capita food fish supply was estimated at 13.7 kg excluding China. In 2007, fish accounted for 15.7 % of the global population's intake of animal protein and 6.1 % of all protein consumed. Globally, fish provides more than 1.5 billion people with almost 20 % of their average per capita intake of animal protein and 3.0 billion people with at least 15 % of such protein. In 2007, the average annual per capita apparent fish supply in developing countries was 15.1 kg, and 14.4 kg in low-income food-deficit countries.

World population has been increasing rapidly during the last few decades with increased exploitation of protein resources. Aquaculture industry remains a growing, exciting and vital production sector to meet a chief protein food requirement. Aquaculture accounted for 46 % of total food fish supply. The FAO (FAO 2012) has reported that about 35 million peoples are fishing around the world with 20 million boats. About 170 million jobs depend directly or indirectly on the fisheries sector, bringing the total web of people financially linked to 520 million. As of 2008, the value of the world aquaculture harvest, excluding aquatic plants, was estimated at US$ 98.4 billion. Average annual per capita supply of food fish from aquaculture for human consumption has increased by ten times, from 0.7 kg in 1970 to 7.8 kg in 2008, at an average rate of 6.6 % per year (FAO 2010). The average per

capita fish consumption is expected to increase to a level of 11 kg per annum for fish eating population.

The global fish production is 154 million tones more or less equally contributed by capture and culture methods (FAO 2012), with major contribution (80 %) from inland aquaculture besides marine capture fishery. However, the wild catches are getting diminishing due to over exploitation, climate change and pollution. The UNEP-Green Economy preview report stated that if the world remained on its path of overfishing, by 2050 the ocean fish stock could become uneconomic to exploit or extinct. Under these circumstances, aquaculture is considered to be a promising sector to fulfill our protein requirement. Marine and brackishwater aquaculture sector is very important because it contributes to food and nutritional security, employment, supports for livelihoods, and which raises the socioeconomic status of poor fishing communities. Hence, aquaculture production should be increased to achieve high yield to eradicate the hunger, malnutrition and poverty of our teeming millions. Therefore, the present book which is the compilation of research works in diverse fields would be immensely useful to the students, researchers and fish farmers.

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References

FAO (2010) The state of world fisheries and aquaculture. FAO, Rome, 197 pp
FAO (2012) The state of world fisheries and aquaculture. FAO, Rome, 209 pp

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About the Editors

Dr. Thirunavukkarasu A.R. is a Retired Principal Scientist and Head, Fish Culture Division, Central Institute of Brackishwater Aquaculture (CIBA), Indian Council of Agricultural Research, Chennai, Tamil Nadu, India. He obtained his M.Sc. and Ph.D. Degrees in Marine Biology from the C.A.S. in Marine Biology, Annamalai University. He started his career as a Research Assistant in the Tamil Nadu State Fisheries Department and then joined as a scientist in the Central Marine Fisheries Research Institute (Govt. of India), where he served till 1991 and subsequently moved to the CIBA. His specialization include Marine Fisheries Management, Inland Fisheries Development and Brackishwater Aquaculture. He has about three decade research experience with over 200 publications including manuals, edited books besides symposium proceedings. He has successfully completed over 20 major research projects worth to the tune of Rs. 8-crore, funded by Indo-French scheme, NFDB, NAIP, DBT, NATP, Mega Seed Bank, etc. To the pinnacle of his achievements, during mid-nineties, he has developed a new technique for the induced breeding and seed production in the commercially valuable finfish, Asian Seabass-*Lates calcarifer* that has revolutionized the Indian Aquaculture industry with the year round sustained supply of seeds. The Seabass seed production technology was transferred to the Rajiv Gandhi Centre for Aquaculture (RGCA), Marine Products Export Development Authority (MPEDA – Govt. of India) and he has been actively involved in the consultancy services to the private agencies like M/S Seabass Hatchery and to the aqua-culturists at large. Subsequently, his research team made a technological break-through for viable land-based brood stock production of Cobia *Rachycentron canadum* in pond and RCC tank holding systems. Further, he has successfully standardized the techniques and developed protocols for the controlled breeding of Grey mullet (*Mugil cephalus*) through pond reared stock, for the development of land-based broodstock of brackishwater ornamental fishes Scat (*Scatophagus argus*) and Moon fish (*Monodactylus argenteus*), controlled breeding and hormone-based sex reversal in Grouper (*Epinephelus tauvina*), maintenance of captive broodstock of Milkfish (*Chanos chanos*), etc. Under the NATP Scheme, he has standardized culture technique for the controlled breeding and seed production of shrimps in inland saline water environs. He is the Chairman of Steering Committee for evaluating the status of Inland Fish seed

Production, Adviser to the Tamil Nadu government-Task Force on Fisheries Development besides an expert member in many universities and national level scientific committees. He was conferred with Gold Medal Award and Citation by the Aquaculture Foundation of India, Chennai and Hiralal Chowdhry Award by Fishing Chimes. He has visited several countries like Norway, Vietnam, France and Thailand.

Dr. Perumal Pachiappan is a Professor and Head, Department of Biotechnology, Periyar University, Salem, Tamil Nadu, India. Prior to his appointment in Periyar University, he had served as a Faculty-Research Associate, Lecturer, Reader and Professor in the C.A.S. in Marine Biology, Annamalai University, where he received his Ph.D. Degree in 1989. He is also qualified in UGC-National Educational Test, 1985. He has over 30 years of research experience with about 150 publications to his credit. His specialization includes molecular taxonomy and live feed aspects of plankton, fish larviculture, and antibiotic principles/drugs from the marine bioresources. He has about 25 years of PG-teaching experience, has successfully guided over 12 Ph.Ds, about 40 P.G. Dissertations and currently guiding many Ph.Ds, M.Phils and M.Sc. students. He has successfully completed many major research projects funded by ICAR, MoEs, DRDO (Govt. of India) and is currently operating a major research project funded by the UGC. He is a member of Academic Council, Senate, Board of Research Studies (of Periyar University), Chairman of PG-Board of Studies in Biotechnology and Bioinformatics (of Periyar University) besides member of Board of Studies in various colleges and universities. He is the Chairman of the Periyar University-Institutional Animals Ethical Committee and Co-ordinator of the Periyar University-Patent Facilitation Centre and Industrial MOU, an expert-member for Projects Review committee of European Commission (EC), Scientist-Member of Govt. Mohan Kumaramangalam Medical College – Institutional Animal Ethics committee and Scientific Advisory committees besides serving as reviewer/editor for many national and international journals. He is a widely travelled man and has visited countries like China, Singapore, Korea, Thailand, France, Spain and Scotland.

Dr. Santhanam Perumal is an Assistant Professor in the Department of Marine Science, School of Marine Sciences, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India. He obtained his M.Sc., M. Phil. and Ph.D. Degrees in Marine Biology, from the C.A.S. in Marine Biology, Annamalai University. He has over 18 years of research and 8 years of teaching experience with over 90 publications in national and internationally reputed journals, books and proceedings. He has been specializing in the areas of Marine Planktonology and Aquaculture with reference to biodiversity, taxonomy, biology and culture of live feeds (marine microalgae and marine copepods) for fish larviculture. He has successfully guided several PG (35), M. Phil. (12) and Ph.D. (6) students and currently guiding many research scholars. He has successfully completed many major research projects funded by UGC, DST and DBT (Govt. of India) and is currently

operating 2 major research projects funded by the DST and DBT (Govt. of India). He is a member in several scientific bodies and has been serving as editorial board member for over 4 journals and reviewer for over 20 reputed national and international journals. He has received DST-Young Scientist Award in 2007 and has been expert-member for various committees.

Isolation and Culture of Microalgae

Perumal Pachiappan, B. Balaji Prasath, Santhanam Perumal, S. Ananth, A. Shenbaga Devi, S. Dinesh Kumar, and S. Jeyanthi

Introduction

Marine microalgae or phytoplankton are the floating microscopic unicellular plants of the seawater which are generally free living, pelagic with the size range of 2–20 µm. The important components of microalgae are the diatoms, dinoflagellates, green algae, blue-green algae, and coccolithophores. Most microalgae have got immense value as they are rich sources of essential fatty acids, pigments, amino acids, and vitamins. They play a critical role in the coastal aquaculture of fish, molluscs, shrimps, and oysters, especially to meet the nutritional requirement of the larvae as well as for bioencapsulation. It is an established fact that the success of any hatchery operation mainly depends on the availability of the basic food, the phytoplankton. The maintenance and supply of the required species at appropriate time form a major problem being encountered by the algal culturists. The procedure for the phytoplankton culture involves aspects such as the isolation of the required species, preparation of the suitable culture media, and maintenance of the culture in the laboratory scale, as well as large scale under controlled conditions of light, temperature, and aeration, and their constant supply to the aqua farmers in different phases of growth. A culture may be defined as an artificial environment in which the microalgae grow. The culture of phytoplankton is an important aspect of planktonology, and the mass culture of phytoplankton is achieved under laboratory-controlled conditions and under field/outdoor conditions. Under laboratory conditions, sterilized or thoroughly cleaned containers are filled with filtered/sterilized seawater (28–34‰) and enriched with the addition of fertilizers, i.e., Guillard and Ryther's F medium, Walne's medium, or TMRL medium. The culture containers are inoculated with pure strains of the desired phytoplankton previously cultured in the laboratory. They are provided with heavy aeration and light using aerators and fluorescent bulbs respectively in a controlled laboratory with temperature of 25 ± 2 °C. The exponential growth phase is generally observed in 36 h to 3 days after inoculation. Cell density of 1.5–4.5 million cells per ml could be recorded. As a sufficient quantity of phytoplankton inoculums usually is present in the coarsely filtered seawater when the nutrients are added, a phytoplankton bloom develops in a course of few days under substantial sunlight. However, it happens sometimes that diatom bloom is inhibited by lack of sunlight or due to the nature of seawater in the tank. In such cases, the addition of new seawater and/or addition of ferric chloride in small amounts may stimulate instant resumption of the diatom in culture.

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History of Microalgae Culture

Microalgae culture is one of the methods of modern biotechnologies. The first unialgal culture was achieved by Beijerinck in 1890 with *Chlorella vulgaris*, and the use of such cultures for studying plant physiology was developed by Warburg in the early 1900s (Warburg 1919). Mass culture of microalgae really began to be a focus of research after 1948 at Stanford (USA), Essen (Germany), and Tokyo, and the classic book edited by Burlew (1953) summarizes many of these early studies. Interest in applied algal culture continued, especially with studies on the use of algae as photosynthetic gas exchangers for space travel and as microbial protein sources. Commercial large-scale culture of microalgae commenced in the early 1960s in Japan with the culture of *Chlorella* followed in the early 1970s with the establishment of a *Spirulina* harvesting and culturing facility in Lake Texcoco, Mexico, by Sosa Texcoco S. A. In 1977, Dainippon Ink and Chemicals Inc. established a commercial *Spirulina* plant in Thailand, and by 1980 there were 46 large-scale factories in Asia producing more than 1,000 kg of microalgae (mainly *Chlorella*) per month (Kawaguchi 1980), and in 1996 about 2,000 t of *Chlorella* were traded in Japan alone. Other *Spirulina* plants were established in the USA (e.g., Microbio in California and Cyanotech in Hawaii). Commercial production of *Dunaliella salina*, as a source of β-carotene, became the third major microalgae industry when production facilities were established by Western Biotechnology Ltd and Betatene Ltd (now Cognis Nutrition & Health) in Australia in 1986. These were soon followed by other commercial plants in Israel and the USA. As well as these algae, the large-scale production of cyanobacteria (blue-green algae) commenced in India at about the same time. More recently, several plants producing *Haematococcus pluvialis* as a source of astaxanthin have been established in the USA and India. Thus, in a short period of about 30 years, the industry of microalgal biotechnology has grown and diversified significantly.

Role of Microalgae in Aquaculture

Microalga is an important source of nutrition and is used widely in the aquaculture of other organisms, either directly or as an added source of basic nutrients. Aquaculture farms rearing larvae of molluscs, echinoderms, crustaceans, and fish use microalgae as a source of nutrition. Low-bacterial and high-microalgal biomass is a crucial food source for shellfish aquaculture.

Microalgae can form the start of a chain of further aquaculture processes. For example, a microalga is an important food source in the aquaculture of brine shrimp. Brine shrimp produces dormant eggs, called cysts, which can be stored for long periods and then hatched on demand to provide a convenient form of live feed for the aquaculture of larval fish and crustaceans.

Other applications of microalgae within aquaculture include increasing the aesthetic appeal of finfish bred in captivity. One such example can be noted in the aquaculture of salmon, where a microalga is used to make salmon flesh pinker. This is achieved by the addition of natural pigments containing carotenoids such as astaxanthin produced from the microalga *Haematococcus* to the diet of farmed fish.

Methods of Isolation of Microalgae Single Species

The isolation of the required species can be done by one of the following methods:

Washing method or centrifugation: Repeated washing or centrifuging the water samples results in the isolation of larger organisms.

By exploiting the phototactic movement: By this method, the phytoflagellates will move to one direction and with a micropipette can be isolated.

By agar plating method: For preparing the agar medium, 1.5 % agar is added to 1 L of suitable medium or even natural seawater, and this agar solution is sterilized in an autoclave for 15 min under 150 lbs pressure and 120 °C temperature. Then this medium is poured in

sterilized Petri dishes and left for 24 h. In case of culture tubes, the medium is poured in 1/3 part in tubes and properly plugged with cotton before autoclaving.

Micromanipulation: The algal cell is to be isolated in drop of enrichment sample. While observing, the cell is sucked up into micropipette. The cell is transferred to a drop of sterile medium on agar plate. This process is repeated to "wash" the cell. The more times a cell is washed, the less likely is bacterial contamination. However, the risk of cell damage increases with the number of times a cell is handled. The optimum number of washes will depend on the type of algae. Then transfer the cell to dilute medium in a tissue culture plate, Petri dish, or culture tube. Culture vessel is placed under low light at appropriate constant temperature. Growth is checked under the microscope, or we have to wait until macroscopic growth can be detected (3–4 weeks after transfer). A colonial unialgal culture results from this method.

Serial dilution: Tubes have to be labeled as 10^{-1}–10^{-10} to indicate dilution factor. Aseptically 1 ml of enrichment sample is to be added to the test tube (10^{-1}) and mixed gently. 1 ml of this dilution is taken and added to the next tube (10^{-2}) and then mixed gently. This procedure is repeated for the remaining tubes (10^{-3}–10^{-10}). Test tubes are incubated under controlled temperature and light conditions: The cultures are examined microscopically after 2–4 weeks by withdrawing a small sample aseptically from each dilution tube. A unialgal culture may grow in one of the higher-dilution tubes, e.g., 10^{-6}–10^{-10}. If the tubes contain two or three different species, then micromanipulation can be used to obtain unialgal cultures.

Growth Dynamics

The growth of an axenic culture of microalgae is characterized by five phases. Growth usually refers to changes in the culture rather than changes in an individual organism. Growth denotes the increase in number beyond that present in the original inoculums. Five distinct phases of growth are described (Fig. 1):

The lag phase – After the addition of inoculums to a culture medium, the population remains temporarily unchanged. The cells at this point increase in size beyond their normal dimensions. Physiologically, they are very active and are synthesizing new protoplasm. The organisms are actually metabolizing, but there is a lag in cell division.

The logarithmic or exponential phase – The cells begin to divide steadily at a constant rate. Given optimum culture conditions, growth rate is maximal at this stage.

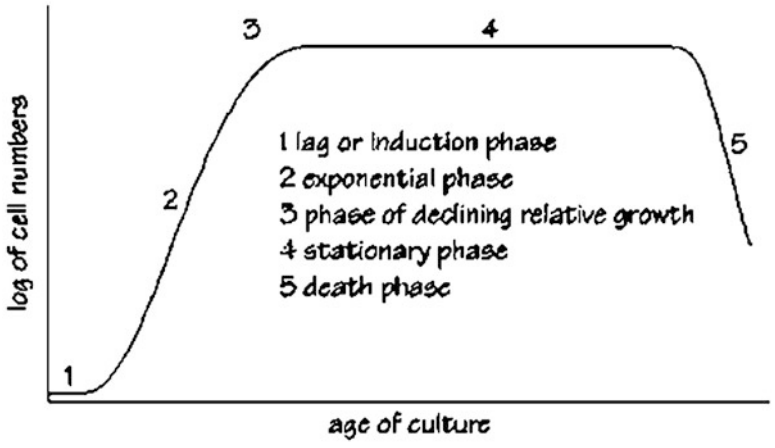

Fig. 1 Schematic diagram of algal growth stages

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.

The stationary phase – At this point, the logarithmic phase of growth begins to taper off after several hours (or days) in a gradual fashion. The population more or less remains constant for a time, perhaps as a result of complete cessation of division or the balancing of reproduction rate by an equivalent death rate.

The phase of decline or death – The rate at which some cells die is faster than the rate of reproduction of new cells. The number of viable cells decreases geometrically.

Materials Required for Culture of Marine Microalgae

Culture Apparatus

Vessels made up of "Pyrex" or "Corning" glass are usually used for phytoplankton culturing. But from test tube to concrete tanks may be used, depending on the quantity of the culture required. For small-scale experiments, Erlenmeyer flasks equipped with inlet and outlet tubes for aeration are used. Glass tubes or a flask plugged with cotton provides enough aeration. The vessels should be cleaned well and sterilized in a hot air oven.

Light

As with all plants, microalgae 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 sunlight, small container close to artificial light) may result in photoinhibition. Also, overheating due to both natural and artificial illumination should be avoided. The duration of artificial illumination should be minimum 18 h of light per day, although cultivated phytoplankton develop normally under constant illumination. In controlled rooms, white daylight fluorescent lamps may be used. Outdoor cultures rely on sunlight for illumination.

Temperature

Temperature usually affects an organism's metabolic rate. Low temperatures are usually maintained in controlled rooms (18–23 °C). Transfer of algal starters or inoculate previously cultured in controlled rooms when scaled up for mass production should be done early morning to avoid stress brought about by sudden temperature increase. 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 the strain cultured. Most commonly cultured species of microalgae 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.

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. 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.

Aeration/Mixing

Mixing is necessary to prevent sedimentation of the algae, to ensure that all the 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, Erlenmeyer), aerating (bags, tanks), or using paddle wheels and jet pumps (ponds). However, it should be noted that not all algal species can tolerate vigorous mixing.

Carbon Dioxide

Providing the algae with extra carbon, in the form of the gas carbon dioxide (CO_2), would facilitate much faster growth. CO_2 is supplied from compressed gas cylinders, and only very little is needed (about half of one percent) in the air supplied to the culture. The CO_2 has to be passed through a flowmeter to ensure that the amount used will keep the pH of the culture between 7.8 and 8.0. The pH can be checked with indicator papers, which change color with a change in pH, or with a pH meter. Both the air and the CO_2 should be filtered through an in-line filter unit of 0.3–0.5 μm before entering the culture, as this helps to prevent other, possibly contaminating, organisms from getting into the cultures.

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 seawater with tap water. Salinities of 20–24 $g.l^{-1}$ have been found to be optimal.

Nutrient Medium

In laboratory cultures, however, natural waters themselves are unsatisfactory for sustained algal growth mainly because some essential nutrients are usually present only in trace amounts. The concentration of these elements largely depends on dynamic equilibrium which is disturbed as soon as water is collected. Miquel (1890–93) observed that the waters of lakes, ponds, and the sea could not support in the laboratory continued and luxuriant growth of algae. Natural waters has to be enriched by the addition of some mineral salts that compounded in the famous solutions A and B. This marked the beginning of the use of enriched culture media where specific conditions are imposed to encourage growth of particular organisms. After the work of Allen and Nelson (1910) on Miquel's solutions, the newly formulated Allen-Nelson "Miquel seawater" medium became the standard for enriching seawater. Synthetic or artificial media were later developed for studies related to algal physiology and nutrition. Provasoli et al. (1957) have observed that artificial media show the most constant results for algal culture in contrast to enriched seawater media which may show varying results depending upon the time and place of collection of the seawater base. Some observations (Chu 1942), however, showed that although the heaviest cultures obtained have been in defined media, frequently growth fails

in it and more often stops at a much earlier stage than in media containing natural materials. In the years that followed, the general trend was toward the improvement of the currently existing synthetic media and the introduction of new ones. 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. 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. Several enrichment media that have been used extensively and are suitable for the growth of most algae are the Walne's medium and the Guillard's F/2 medium. Various specific recipes for algal culture media are described by Vonshak (1986). These recent advances in algal culture somehow present probable answers to the many problems in aquaculture.

Selection of the Culture Medium

On securing the desired organism, microalgal sample to be transfer into a series of Petri dishes, each containing different enriched media. They have to be exposed to sunlight or artificial light. This preparatory culture is used to select the suitable medium for the particular species. During this time, the organism multiplies in one of the media and provides enough material for further process of culturing. Pure cultures are sometime obtained only after several attempts. The preparatory cultures may be maintained till pure cultures are obtained.

Culture Media

The following are some of the culture media found suitable to most planktonic algae:
TMRL medium (Tung Kang Marine Res. Lab.)

Potassium nitrate	10 g/100 ml of DW
Sodium orthophosphate	1 g/100 ml of DW
Ferric chloride	0.3 g/100 ml of DW
Sodium silicate	0.1 g/100 ml of DW

The chemicals are kept separately in 100 ml reagent bottle. 1 ml each to 1 l of sterilized seawater is added. This medium can be used for the mass culture of diatom.

Schreiber's medium

Potassium nitrate	0.1 g
Sodium orthophosphate	0.02 g
Soil extract	50 ml
Filtered and sterilized seawater	1

Soil extract is prepared by boiling 1 kg of garden soil in 1 l of fresh water for 1 h. After 24 h, clear water is decanted and kept in a bottle. 50 ml of this soil extract can be added to each liter of sterilized seawater. This can be used as a medium while isolating the nanoplankton.

F/2 medium

$NaNO_3$ (75.0 g/L dH_2O)	1.0 ml
$NaH_2PO_4 \cdot H_2O$ (5.0 g/L dH_2O)	1.0 ml
$Na_2SiO_3 \cdot 9H_2O$ (30.0 g/L dH_2O)	1.0 ml
F/2 trace metal solution	1.0 ml
F/2 vitamin solution	0.5 ml
Filtered seawater to	1.0 L

After all additions, the medium will be autoclaved.

F/2 trace metal solution

$FeCl_3 \cdot 6H_2O$	3.15 g
$Na_2EDTA \cdot 2H_2O$	4.36 g
$CuSO_4 \cdot 5H_2O$ (9.8 g/L dH_2O)	1.0 ml
$Na_2MoO_4 \cdot 2H_2O$ (6.3 g/L dH_2O)	1.0 ml
$ZnSO_4 \cdot 7H_2O$ (22.0 g/L dH_2O)	1.0 ml
$CoCl_2 \cdot 6H_2O$ (10.0 g/L dH_2O)	1.0 ml
$MnCl_2 \cdot 4H_2O$ (180.0 g/L dH_2O)	1.0 ml
Distilled water to	1.0 L

F/2 vitamin solution

Vitamin B_{12} (1.0 g/L dH_2O)	1.0 ml
Biotin (0.1 g/L dH_2O)	10.0 ml
Thiamine HCl	200.0 mg
Distilled water to	1.0 L

Filter sterilizes into plastic vials, and stored in the refrigerator.