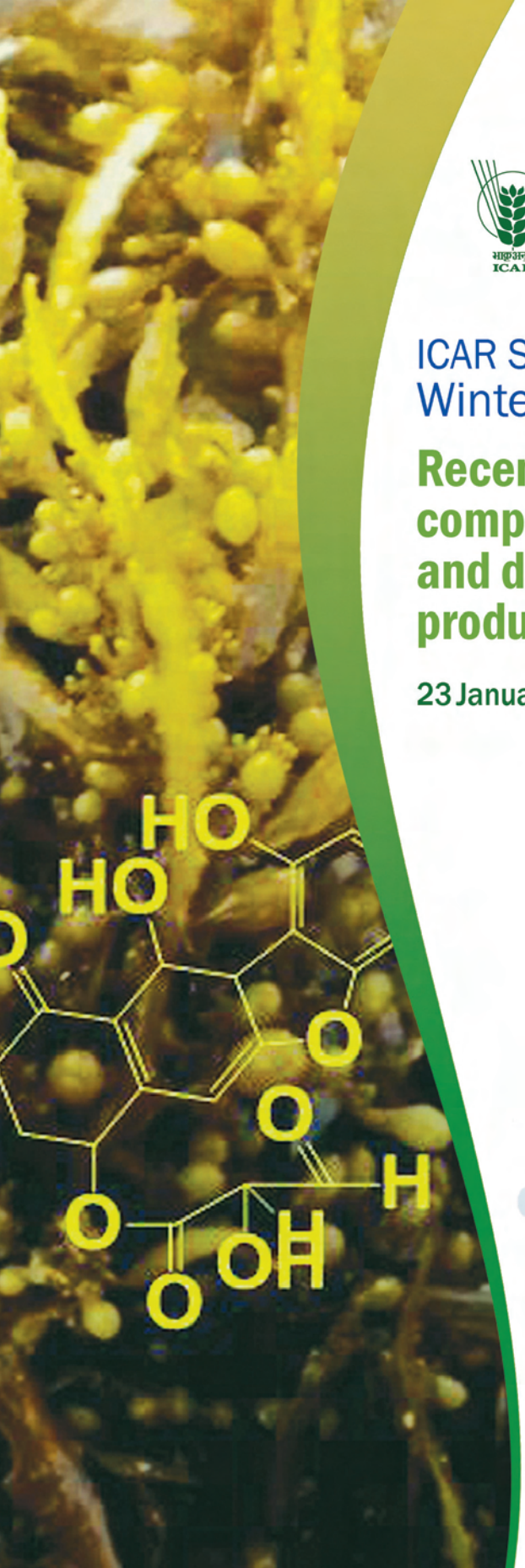
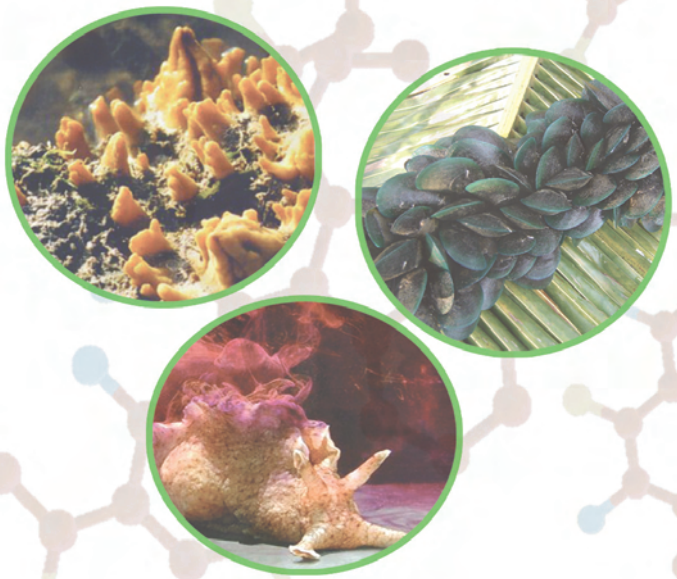
CMFRI Training Manual Series No.13/2018



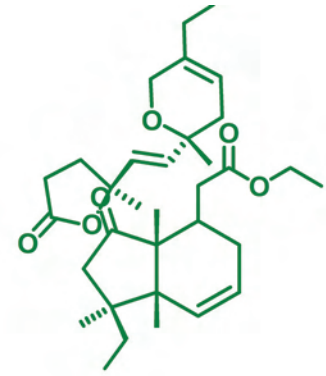
ICAR Sponsored Winter School on

Recent advances in bioactive compounds from marine organisms and development of high value products for health management

23 January to 12 February 2018



Marine Biotechnology Division
ICAR-Central Marine Fisheries Research Institute
Post Box No. 1603, Ernakulam North P.O., Kochi-682 018, Kerala, India



ICAR Sponsored
Winter School on

Recent advances in bioactive compounds from marine organisms and development of high value products for health management

23 January to 12 February 2018

Marine Biotechnology Division
ICAR-Central Marine Fisheries Research Institute
Post Box No. 1603, Ernakulam North P.O., Kochi-682 018, Kerala, India

Course Manual

ICAR-Winter School on

Recent advances in bioactive compounds from marine organisms and development of high-value products for health management

CMFRI Training Manual Series No. 13/2018
ICAR-Central Marine Fisheries Research Institute,
23 January - 12 February, 2018

Publisher :

A. Gopalakrishnan
Director
ICAR-Central Marine Fisheries Research Institute
Ernakulam North P. O., Kochi - 682 018, Kerala

Compilation :

Kajal Chakraborty

Minju Joy

Technical Assistance :

Minju Joy
Soumya Krishnan
Vinaya K. K.
Prima Francis
Subhajit Dhara
Aswathy Elizabeth Mani
Tima Antony
Soumya Salas
Sreemol C. K.

Course Director :

Kajal Chakraborty
Senior Scientist
Marine Biotechnology Division

Course Coordinator :

Vijayagopal Pananghat
Head of Division & Principal Scientist
Marine Biotechnology Division

Cover Design :

Abhilash P. R.

This manual has been prepared as a reference material for the ICAR funded Winter School on "Recent advances in bioactive compounds from marine organisms and development of high-value products for health management" held at Central Marine Fisheries Research Institute, Kochi during 23 January - 12 February, 2018

Edited by Foxit Reader

FOREWORD

There has been a growing interest in the marine derived bioactive compounds in the recent years, and the functional foods, enriched with natural ingredients have been proved to provide beneficial action for human health. Marine derived bioactive components and the functional food ingredients demonstrated to possess potential health benefits. High value secondary bioactive metabolites from the marine organisms are attracting attention because of the growing demand for new compounds of 'marine natural' origin, having potential applications in pharmaceutical fields, and concerns about the adverse effects by synthetic drugs and their derivatives. The pioneering R & D works at ICAR-Central Marine Fisheries Research Institute on marine bioprospecting envisaged a systematic approach involving chemical profiling of major species of marine organisms for bioactive pharmacophore leads for activity against various diseases, and a library of molecules with bioactive potential. The research work in this institute developed protocols to prepare various pharmaceutical leads, nutraceuticals/ functional food supplements enriched with lead molecules with different properties against various drug targets for use against various life-threatening diseases.

ICAR-Central Marine Fisheries Research Institute is the pioneering marine research institute in India to work in the frontier area of bioactive molecule discovery from marine organisms as promising therapeutic agents against various diseases, aquatic food product technology, and development of high value products for health management. This prestigious research institute of Indian Council of Agricultural Research is working in the broad national interest of producing high value bioactive leads from the marine organisms, which would provide promising therapeutic agents against various diseases. This institute has developed and commercialized the nutraceutical products Cadalmin™ Green Algal extract (Cadalmin™ GAe) and Antidiabetic extract (Cadalmin™ ADe) as green alternatives to synthetic drugs to combat rheumatic arthritic pains and type-2 diabetes, respectively to a leading biopharmaceutical company in India. The anti-inflammatory nutraceutical Cadalmin™ Green Mussel extract (Cadalmin™ GMe) from Asian green mussel Perna viridis has been commercialized with Amalgam Group of Companies. Cadalmin™ Antihypercholesterolemic extract (Cadalmin™ ACe) has been developed from seaweeds to combat dyslipidemia leading to obesity, and the product was out-licensed to a leading Indian MNC in wellness and obesity management. Antimicrobial therapeutic product from marine bacteria as oral applicant has been developed and the product is in pipeline for commercialization. Seaweed-derived natural template inspired synthetic derivatives as potential pharmacophores were designed and developed. Several nutraceutical and cosmeceutical products from marine organisms are in pipeline, and are being commercialized.

The objective of the National level ICAR Winter School on "Recent advances in bioactive compounds from marine organisms and development of high-value products for health management" is to provide up-to-date information and acquaint the participants with the latest technologies on isolation and characterization of marine natural products of pharmaceutical importance from marine organisms, general and advanced methods of isolation procedures by chromatography, classification of organic compounds and their characterization by advanced spectroscopic experiments. This program further aims to give exposure to the chemical perspectives of marine organisms, primary and secondary bioactive metabolites from fish and marine organisms to develop bioactive compounds and high-value functional food products. Theory and practical classes will be conducted in these areas to provide the participants a hands-on experience.

This ICAR Winter School is organized with the full funding support from ICAR, New Delhi, and the twenty-five participants from various parts of India who are attending this programme were selected after scrutiny of their applications based on their bio-data. They are serving as academicians, such as Professors/Scientists, and in similar posts. The faculties include the knowledgeable scientists and professors from various parts of India and abroad. This training will enable the participants to efficiently carry out their academic programmes, and to plan research on bioactive molecule discovery in their respective laboratories and institutes so that they can formulate the strategies for research.

The Winter School on "Recent advances in bioactive compounds from marine organisms and development of high value products for health management" is very ideal for the current scenario of increasing lifestyle diseases and human health. Understanding the importance of natural products in the health care system of India, ICAR-Central Marine Fisheries Research Institute has reasonably contributed in the various aspects. The Manual released on this occasion covers all aspects of marine natural products prepared by the experts in their respective fields. I congratulate the Course Director of this programme, Dr. Kajal Chakraborty and Head of the Marine Biotechnology Division, Dr. P. Vijayagopal, along with other staff members of Marine Biotechnology Division and Central Marine Fisheries Research Institute for their sincere efforts in bringing out the manual in time, and to arrange the programme in a befitting manner.

A. Gopalakrishnan
Director, ICAR-Central Marine Fisheries Research Institute
Kochi, Kerala

PREFACE

Marine-derived bioactive components and the functional food ingredients with potential health benefits are an emerging area of research. The rich diversity of flora and fauna in the marine and coastal habitats of the Indian subcontinent represent an untapped reservoir of bioactive compounds with valuable pharmaceutical and biomedical use. Considering the underutilization of these groups of marine organisms, exploring bioactive compounds and development of any biologically useful products have benefits as health products. Comprehensive analyses demonstrated that during the last decade the average proportion of bioactive compounds among the new compounds is declining, though there are a large number of marine natural products yet to be explored. This may indicate that the research level of bioactivity is not keeping up with the discovery of new compounds. Thus, the research tools and methods for finding bioactivity need to be improved. The first improvement is about methods of spectral and bioactivity-guided separation and purification of marine-derived secondary metabolites, which combine the discovery of new compounds. These improvements in technology are dependent upon the automation in spectroscopy, which also allows the study of the functions of new compounds extracted from the target marine organisms. Second, for the discovery of new lead compounds and artificial intelligence for drug development evolved to a more mechanistic approach that targets specific molecular lesions. Combined with high-throughput screening through a large number of drug targets, bioactivity research against various life-threatening diseases will be effective in revealing the potentially useful biological properties of marine natural products. Furthermore, the discovery of new bioactive compounds from marine metabolites will form the basis for new drug leads. Thus, the new compounds will absolutely compose an abundant resource for future bioactivity research and drug development. Various medicinal and biomedical products from marine flora and fauna provide a myriad of benefits for human health and multiple life-threatening diseases, and therefore, are the attractive options for the food and pharmaceutical industry. The increasing interest in marine-based functional food ingredients and nutraceutical formulations in the last decade along with increased number of patents filed/granted have appropriately demonstrated the possibilities of bioactive from marine organisms to maintain and improve human health and well-being.

The present ICAR Winter School on "Recent advances in bioactive compounds from marine organisms and development of high-value products for health management" is designed to acquaint the participants with the advances in marine bioactive compounds with emphasis on the latest technologies on isolation and characterization of marine natural products of pharmaceutical importance. The course is planned in such a way that it covers both theoretical and practical aspect of recent advances in bioactive compounds from marine organisms. This programme will strengthen the knowledge of participants with regard to

the general and advanced methods of isolation procedures by chromatography, and their characterization by advanced spectroscopic experiments aspects.

I wish to thank the Education Division of Indian Council of Agricultural Research for giving us an opportunity to organize this ICAR Winter School. We are grateful to Dr. A. Gopalakrishnan, Director, ICAR-Central Marine Fisheries Research Institute, for his guidance, continuous interest in the course and providing all necessary facilities. I am highly obliged to Dr. P. Vijayagopal, Head, Marine Biotechnology Division for his guidance and support for the programme. All the scientists of Marine Biotechnology Division, technical staff, supporting staff and research scholars supported us in organizing the ICAR Winter School. I recall with gratitude the marvellous effort and help in preparing this manual by Minju Joy, Research Scholar of Marine Biotechnology Division. I take this opportunity to thank all the faculty members who have devoted their valuable time and contributed material for the preparation of the manual. I am confident that the Course Manual would aid the participants to enhance their knowledge and competence in the area of marine bioactive compounds and their applications for the development of high-value products for health management.

Kajal Chakraborty
Course Director

January, 2018

CONTENTS

Chapter	Topic	Page
1	MARINE ORGANISMS: THE UNDEREXPLORED RESOURCES TO DEVELOP HIGH VALUE COMPOUNDS AND THERAPEUTIC PRODUCTS *A. Gopalakrishnan*	**1**
2	MARINE NATURAL PRODUCTS: A FUNCTIONAL FOOD PERSPECTIVE *P. Vijayagopal*	**14**
3	MARINE ORGANISMS-TREASURE HOUSE OF VALUABLE PRODUCTS AND THEIR CHEMICAL PERSPECTIVES *Kajal Chakraborty, Minju Joy, Soumya Salas, Soumya Krishnan*	**30**
4	CLASSIFICATION OF MARINE NATURAL PRODUCTS - CHEMISTRY AND BIOACTIVITY *Kajal Chakraborty, Soumya Salas, Minju Joy, Prima Francis, Subhajit Dhara*	**61**
5	INTRODUCTION TO NATURAL PRODUCTS *Dr. Meledath Govindan*	**82**
6	BIOACTIVE MARINE NATURAL PRODUCTS - A REVIEW *Dr. Meledath Govindan*	**94**
7	NATURAL PRODUCTS: ISOLATION, SEPARATION AND PURIFICATION *Dr. Meledath Govindan*	**108**
8	SPECTROSCOPIC METHODS TO CHARACTERIZE BIOACTIVE COMPOUNDS: NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY *Dr. Meledath Govindan*	**116**
9	INFRARED AND MASS SPECTROSCOPY *Dr. Meledath Govindan*	**128**
10	RECENT TRENDS IN MARINE NATURAL PRODUCTS DISCOVERY PROCESS: CHEMICAL BIOLOGY AND DEREPLICATION *Dr. Meledath Govindan*	**149**

Chapter	Topic	Page
11	SPECTROSCOPIC METHODS TO CHARACTERIZE BIOACTIVE COMPOUNDS: MASS SPECTROSCOPY *Dr. Meledath Govindan*	**160**
12	PHOTOSENSITIZERS AND PHOTODYNAMIC ANTIMICROBIAL CHEMOTHERAPY *Abdulaziz Anas*	**169**
13	NEW WEAPONS TO FIGHT BACTERIAL BIOFILMS IN HEALTH CARE *Rajendran N.*	**178**
14	MARINE MICROBES AS A SOURCE OF ANTIMICROBIAL COMPOUNDS *Kajal Chakraborty, Vinaya K.K., Tima Antony, Minju Joy, Sreemol C.K.*	**189**
15	X-RAY DIFFRACTION: ANALYSIS TECHNIQUES *Shibu M. Eappen*	**199**
16	SAFETY AND HAZARDS IN A CHEMICAL LABORATORY *Kajal Chakraborty, Minju Joy, Soumya Krishnan, Vinaya K. K.*	**204**
17	MARINE NANOPARTICLES AND ITS APPLICATIONS *Anu Gopinath*	**224**
18	RNA TARGETING BY ANTIBIOTIC MIMETICS *Franklin J.*	**230**
19	RECENT ADVANCES OF PREPARATIVE CHROMATOGRAPHY *Dr. Ajit Datar*	**233**
20	HYPHENATED TECHNIQUES: LC-MS *Dr. Ajit Datar*	**240**
21	FUNDAMENTALS OF SPECTROSCOPIC TECHNIQUES WITH REFERENCE TO FTIR *Anu Gopinath*	**259**
22	BIOACTIVE COMPOUNDS FROM MARINE ORGANISMS INCLUDING BACTERIA *Sarita G. Bhat, M. Chandrasekaran*	**268**
23	NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY (PROTON-NMR) *Anu Gopinath*	**274**

Chapter	Topic	Page
24	BIOACTIVE PROTEINS AND PEPTIDES FROM MARINE MICROORGANISMS *Manzur Ali P. P., Sapna K. K., Rakhamol K. R.*	**287**
25	SOLID PHASE SYNTHESIS OF PEPTIDES AS LIGANDS OF NANOPARTICLES FOR BRAIN DRUG DELIVERY *Jaya T. Varkey*	**292**
26	RECENT ADVANCES IN MARINE NATURAL PRODUCTS ISOLATION *T.P. Sajeevan*	**300**
27	CHIRAL MOLECULES FROM RENEWABLE RESOURCES AND THEIR APPLICATION *Grace Thomas*	**307**
28	THEORETICAL BACKGROUND OF COMPUTATIONAL CHEMISTRY *Abi T. G.*	**312**
29	NEW GENERATION ANTI CANCER DRUG UTILIZING MARINE BIOCOMPATIBLE RESOURCES *Jinu George*	**320**
30	CORALS AND SPONGES: IMPORTANT RESOURCE BASE OF BIOACTIVE COMPOUNDS *K. Vinod*	**323**
31	ADVANCES IN ALGAL BIOTECHNOLOGY AND BIOFUEL DEVELOPMENT *Valsamma Joseph*	**328**
32	MINING GENOMES FOR NOVEL BIOACTIVE COMPOUNDS *Toms C. Joseph and K. V. Lalitha*	**343**
33	CLINICAL TRIAL OF BIOACTIVE MOLECULES *K. Gopakumar*	**349**
34	ANIMAL MODELS FOR THE EVALUATION OF BIOACTIVE COMPOUNDS IN CANCER AND PRECEPTFOR THE ETHICAL USE OF ANIMALS IN CANCER RESEARCH *Bibu John Kariyil*	**358**
35	NATURAL PRODUCT INSPIRED SYNTHESIS OF BIOACTIVE COMPOUNDS *Krishnakumar K. S.*	**363**

Chapter	Topic	Page
36	BRYOZOA - TAXONOMY AND DIVERSITY: A POTENTIAL SOURCE OF MARINE BIOACTIVE MOLECULES *Nandini Menon N.*	**373**
37	BIOLOGICAL, TOXICOLOGICAL AND CLINICAL EVALUATION OF BIOACTIVE PHARMACEUTICAL LEADS WITH REFERENCE TO CANCER *Ramadasan Kuttan*	**380**
38	MARINE MICROALGAE: CULTURE AND THEIR INDUSTRIAL APPLICATIONS *K. Madhu, Rema Madhu, Suji Chandru, M. T. Vijayan and M. P. Mohandas*	**384**
39	MARINE BIODIVERSITY: AN IMPORTANT RESOURCE BASE TO DEVELOP BIOACTIVE COMPOUNDS FOR HEALTH AND DISEASES *K. K. Joshi, Sethulakshmi M., Sheeba K. B., Thobias P. Antony and Varsha M. S.*	**392**

CHAPTER

38

MARINE MICROALGAE: CULTURE AND THEIR INDUSTRIAL APPLICATIONS

K. Madhu, *Rema Madhu, Suji Chandru, M. T. Vijayan and M. P. Mohandas

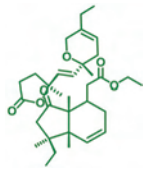
Mariculture Division, ICAR-Central Marine Fisheries Research Institute, Kochi

Microalgae such as *Chaetoceros gracilis*, *C. calcitrans*, *Chlorella salina*, *C. marina*, *Chromulina pleoides*, *Dunaliella tertiolecta*, *Isochrysis galbana*, *Nannochloropsis oculata*, *Pavlova lutheri*, *Skeletonema costatum*, *Tetraselmis chuii, Thalassiosira* sp., are microscopic unicellular phytoplankton having size less than 10 µ, and have widely used in the aquaculture industry. The selection of these live feed organisms are based on many factors such as nutritional requirements of the cultured larvae, size of the mouth gape of larvae, development of the digestive tract of the cultured larvae, nutritional value and availability of the live feed and suitability for mass scale production. Though much efforts are been taken world-wide to supplement live feed totally or partially with artificial feeds, various studies pointed out that supply of suitable live feed organisms fortified with vitamins and fats are essential for the successful completion of the larval stages. In the hatchery system, production of microalgae is being done by various methods viz. Batch culture, semi continuous culture and continuous culture. Recent findings showed that bio-enrichment of live feed organisms with various microalgae have a vital role in larval survivability as the larvae require diets with high protein and sufficient amount of essential fatty acids (EPA and DHA). It has been shown that microalgae also contained vitamin (A, E and C), trace minerals and b-and other carotenoids which can affect fecundity, egg quality, hatchability and larval quality. In addition to these many algae are being used for extraction of bio active compounds for drugs, nutritional supplements, biodiesel, etc.

Microalgae being the predominant component of the first tropic level in the aquatic food chain has got immense value as an aquaculture live feed, and as a result the production of unicellular algae has gained importance in several countries due to their wide use as nutrient food in the hatchery seed production of commercially important shell and fin fishes (Benemann, 1992, Muller-Feuga, 2000). Micro algae also have industrial application as it contains various bioactive compounds. The important microalgae are the diatoms, dinoflagellates, silicoflagellates (phytoflagellates), coccolithophores, blue green algae and the 'hidden flora'- the nannoplankters. Among these, cyanophytes, diatoms and phytoflagellates are significant organisms since they form the primary link in the food chain of the sea as well as it contains many bioactive compounds.

NUTRITIVE VALUE OF ALGAE

In the larval feeding systems, microalgae are being selected on the basis of their size,



nutritional value, culture easiness and absence of negative side effects such as toxicity. Their nutritional value shows a great variability not only among different species, but also in genetically different populations of the same species (strains). Among the different microalgae, only very few species are suitable for fishes and provided better results when it fed to organisms and some are reported to be toxic. Some of the microalgae have flagellas (one or two tiny beating hairs) for motility. These micro planktons have been extensively utilized for mass production of zooplanktons such as rotifers, artemia, copepods, etc. Therefore, these micro planktons are need to be isolated, identified, and evaluate their biochemical composition in suitable media and environmental which are inevitable to increase the production of aquatic species. Thus the culture of microalgae become as an inherent part of aquaculture industry.

Green water techniques

Aquaculture important microalgae such as *C. gracilis*, *C. calcitrans, C. salina, C. marina, C. pleoides, D. tertiolecta, I. galbana, N. oculata, P. lutheri*, *S. costatum*, *T. chuii, Thalassiosira* sp., are main constituents for green water which helps to reduce mortality, better fish health, and also enhance visual contrast and light dispersion - i.e. the fish can see better. This improves food detection and location, and reduces "nose bumping" and "head butting syndrome" which leads to bruising and disease. Improved nutritional value of live prey (rotifers, brine shrimp, etc.) in tank which consumes the microalgae. This system can also stabilize the water quality by removing metabolic products and producing oxygen.

IMPORTANCE OF LIVE FEEDS

In marine fish breeding, the larval stages of many fishes are critical due to their small mouth gape and changes in feeding habit. As a result survivability is also very less which research needs thrust for augmenting survival rate. Among the different live feeds, many species of microalgae, diatoms micro algae and micro zooplanktons together plays an important role in larval production system. The selection of these live feed organisms are based on many factors such as nutritional requirements of the cultured larvae, size of the mouth gape of larvae, development of the digestive tract of the cultured larvae, nutritional value, and availability of the live feed and suitability for mass scale production. Though much efforts are being taken worldwide to supplement live feed totally or partially with artificial feeds, various study pointed out that supply of suitable live feed organisms fortified with vitamins and fat are essential for the successful completion of the larval stages.

MICROALGA ISOLATION AND CULTURE

Over dependence for this inevitable plankton from the wild is an unreliable source for commercial seed production due to uncontrollable fluctuations in quality, quantity and to the drawbacks of collecting methods which do not excludes harmful organisms. Moreover,

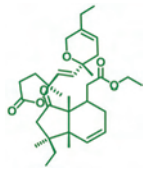
of the very many type of algae which live in the sea, only few can be cultured and certain type only will give good growth. It is therefore, suitable microalgae need to be isolated with different methods such as pipette, centrifuge or washing, phototactic movements, agar plating and serial dilution methods, and the isolated species can be mass cultured in suitable culture media. For the successful culturing of the microalgae, either diatoms or nano plankters, various chemical culture media have been used. While most of the microalgae can be successfully cultured on synthetic inorganic media, a few genera require organic compounds for their rapid growth, and therefore the culture are also supplemented with soil extracts, yeast extracts or organic salts. Since the microalgae in any water body require the nutrients such as nitrates and phosphates roughly in a ratio of 10:1 (N:P) for its normal growth and reproduction, the culture media used in the laboratory culture system should have sufficient quantities of these elements besides other growth promoting agents. The widely used culture media are 'Conway' or Walnes medium Erd- Schreiber's and Miquels TMRL, Suto, PM, SEAFDEC, Gulliard f, f/2,f/4, Johnsons (J/1) ASW, MN,ASPW, etc. and Fresh water micro algal medium are Bold basal, BG-11, PHM-1, Botoryococus and Zarrouk media (Table 1).

Table 1. Microalgae culture media

1. Walnes or Conway medium (For stock culture)

Solution-A			**Solution-B**		
Sl. No.	**Chemicals**	**Quantity**	**Sl. No.**	**Chemicals**	**Quantity**
i.	Pottasiuim Nitrate	100 gm	i.	Zinc Chloride	4.2 gm
ii.	Sodium Ortho-phospate	20 gm	ii.	Cobalt chloride	4.0 gm
iii.	EDTA(Na)	45 gm	iii.	Copper Sulphate	4.0 gm
iv.	Boric acid	33.4 gm	iv.	Ammonium Molybdate	1.8 gm
v.	Ferric Chloride	1.3 gm	v.	D.D.W.	1 lt
vi.	Manganese Chloride	0.36 gm			
Solution-C			**Solution-D**		
Sl. No.	**Chemicals**	**Quantity**	**Sl. No.**	**Chemicals**	**Quantity**
i.	Vitamin B1 (Thiamine)	2000/100 mlDDW	i.	Vitamin B12 (cyanocobalamin)	10 mg/ 100 ml DDW
Solution E					
Sl. No.	**Chemicals**	**Quantity**			
i.	Sodium Meta silicate	4 gm/100 DDW			

Add 1 ml A, 0.5 ml B and 0.1ml C & D to 1 lit. of sea water for all algae for making nutrient media, and for diatoms apart from these also add 2 ml of solution E per 1 lit. of sea water.



STOCK AND MASS CULTURE

Stock or starter cultures are inevitable for the algal culture systems, and these are need to be frequently sub cultured to maintain the culture in the exponential growth phase which is the key factor for the successful and efficient algal production system. The containers for the mass culture of microalgae are of 10-15 l capacity polythene bags/ buckets, 20-1 glass carbuoys, 100-1 perspex tanks and 250 1 cylindrical transparent FRP tanks for the indoor culture. These containers are kept in wooden racks with light and aeration. Fully-grown culture from the stock culture room is used as inoculum for the mass culture in these containers. These tanks have the maximum concentration of the cells in the growing phase on the 5-7 the day and harvested. After estimating the cell concentration using a haemocytomter, the culture is supplied to the hatchery for the rearing operations of the larval organisms. Leaving 2 1 of the culture, fresh enriched medium is added for further culture in the same container. The fully-grown culture should be harvested during the exponential phase of the microalgae after determining the cell concentration.

Media for Mass culture

Solution I (in 4lit)	Solution –II (in 4lit)	Solution –III (1lit)
Pot. Nitrate -1500 gm	Ferric Chloride- 64 gm	B12-1gmThiamine
Sodium ortho	EDTA-(Na)-88gm	Hcl-20gmBiotin-1gm
Phosphate-100gm		

(100 ml make up to 4lt) (*20 ml/ton) (Liquid Silicate-700ml/4000ml)-20ml/ton

MEDIA FOR MASS CULTURE OF ALGAE USING FARM CHEMICALS

Outdoor tanks with 5 to 10 ton can be used for mass culture of algae depending upon the requirements using farm chemicals in the following proportion: adding (per 1000 l of sea water) 1.5 g of urea (NH_2 CON H_2:46% nitrogen), 1.6 g of triple superphosphate (P2 05:19.9% phosphorous) and 10.6 g of sodium metasilicate (NaZ SiO3.5H_2 0:13% silica) will provide the required amounts of nitrogen, phosphorous and silica to stimulate growth and division of algal cells (algal bloom) which will also depends on the temperature of the sea water and the amount of sunshine on the tanks.

PHOTOBIOREACTORS METHOD

Enclosed photobioreactors have been employed to overcome the contamination and evaporation problems encountered in open ponds (Molina Grima et al., 1999). These systems are made of transparent materials and are generally placed outdoors for illumination by natural light. The cultivation vessels have a large surface area-to-volume ratio. The most widely used photobioreactor is a tubular design, which has a number of clear transparent tubes, usually aligned with the sun's rays. The medium broth is circulated through a pump

to the tubes, where it is exposed to light for photosynthesis, and then back to a reservoir. A portion of the algae is usually harvested after it passes through the solar collection tubes, making continuous algal culture possible. In some photobioreactors, the tubes are coiled spirals to form what is known as a helical-tubular photobioreactor (Chisti, 2007).

DETERMINATION OF ALGAL CELL DENSITY

In order to monitor growth of the algal cultures in various culture flasks as well as mass culture tanks, regular counts of the algal cells need to be conducted. Sampling can be done with sterile serological pipette which can be used for dragging the sample. To get the uniform sample, mild agitation with help of sterilized rod to be done and move the pipette around the tank while withdrawing algae up to the mark on the pipette. Samples can be taken from each corner of the tank and then treated with a drop of eosin or 1% formalin to kill the cells and stirred well. Draw sample in pipette and place the tip of the pipette near the V shaped notch of heamocytometer. The sample runs inside the cover slip and thin film of the culture is formed and the cells are equally distributed. In the same way load the second chamber also and allow remaining for 10 minutes. Since the haemocytometer has got 4 grid in 4 corners with 16 divisions in each grid, the counting is restricted to 4 grids at four corners of the haemocytometer chamber. The cell density in 1 ml is calculated as shown below.

Total number of cells ml^{-1} = (Total number of cells in 4 grid x 10^4)/4

If the cell density is in the higher levels (1-25x10^6 ells/ml) count the 8 grids (Both sides) of the haemocytometer

Average No. of cells counted in 8 grid (A) = (Total No. of cells counted)/8

Cell density (cells/ml) = (Ax25)/100 x 10^6 (cells/ml)

If the cell density is greater than 25x10^6 ells/ml, observe the central square of haemocytometer which is divided in to 25 smaller squares, and each of which is further sub divided into 16 sub-squares. Count the number of algal cells in 5 of the 25 squares. Five such squares are counted on each side of the haemocytometer (10 per sample)

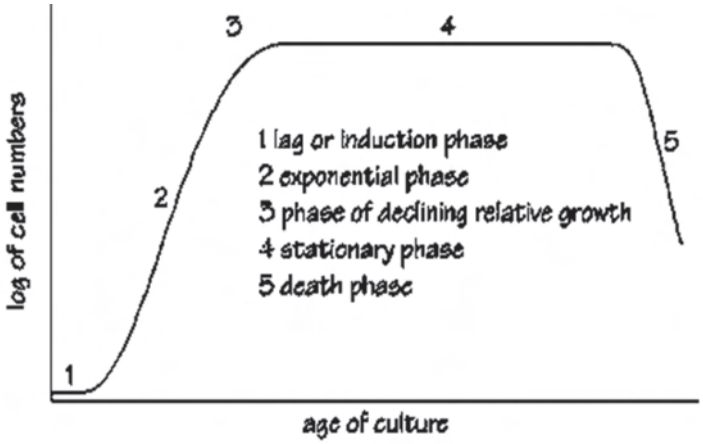
Average No. of cells counted (A) = (Total No. of cells counted)/10

Cell density (cells/ml) = (Ax25)/100 x 10^6 (cells/ml)

GROWTH OF ALGAE

The algal quality is based on nutritive value, size, and cell wall composition and growth characteristics of cultured species. It is also revealed that larvae fed with natural concentration grow more slowly than larvae fed with cultured algae. Thus cultured algae are paramount important live feed in hatchery system for mass production of larvae of crustaceans, molluscs

and fin fishes. The growth of algal culture can be expresses in terms of cell division or doublings per day. Under suitable nutrient enrichment and favourable physical conditions, axenic cultures of algae will exhibit different growth stages: lag-phase or induction phase; exponential phase or log phase; phase of declining relative growth or transitional phase; stationery phase and death phase.



BIO ENRICHMENT

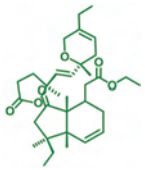
Most of the microalgae are in rich of highly unsaturated fatty acids (HUFAs) including eicosapentaenoic acid (EPA, 20:5n-3), and docosahexaenoic acid (DHA, 22:6n-3). Deficiencies in these lipids result in poor growth, low feed efficiency, anemia and high mortality (Sergent et al., 1999; Olivotto et al., 2003). It is therefore, microalgae are being used as bio-enrichment agent for live food organisms like Artemia and Rotifer possessing low essential fatty acids eicosapentaenoic acid (EPA) and decosa hexaenoic acid (DHA) which influence the reproductive success of all animals including fish, and it has also been shown that essential fatty acids, vitamin (A, E and C), trace minerals and b-and other carotenoids can affect fecundity, egg quality, hatchability and larval quality.

BIOACTIVE COMPOUNDS ISOLATED FROM MICROALGAE

Numerous *in vitro* and animal studies have established the potential colon and skin cancer chemo- preventive properties of substances from marine environment, including microalgae species and their products (carotenoids, fatty acids, glycolipids, polysaccharides and proteins). The actions include suppression of cell proliferation, induction of apoptosis, stimulation of anti-metastatic and anti-angiogenic responses and increased antioxidant and anti-inflammatory activity (Elena et al., 2015) (Table 2). In addition to these, microalgal biotechnology appears to possess high potential for biodiesel production because, significant increase in lipid content of microalgae is now possible through heterotrophic cultivation and genetic engineering approaches (Table 3). Although oils extracted from microalgal cells have been investigated for fuel production of internal-combustion engines by trans-esterification of fatty acids (Pyrolyzing method), industrial biodiesel production from microalga oils is still not well developed.

Table 2. Compounds obtained from microalgae and their biological activities

Compound	Source /algal species	Activity
CAROTENOIDS		
β-Carotene	*Dunaliella salina, Haematococcus* sp.	Antioxidant,Pro-vitamin AAnti-inflammatory, Anticancer
Astaxanthin	*Haematococcus pluvialis, Chlorella zofigiensis, Chlorococcum* sp.	Antioxidant, Anti-inflammatory, Anticancer
Lutein	*Dunaliella salina, Chlorella sorokiniana Chlorella,prothecoides*	Antioxidant, Anti-inflammatory Anticancer
Violaxanthin	*Dunaliella tertiolectaChlorella ellipsoidea*	Anti-inflammatory, Anticancer
Zeaxanthin	*Synechocystis* sp., *Chlorella saccharophila*	Antioxidant, Anti-inflammatory
Fucoxanthin	*Phaeodactylum tricornutumIsochrysis* sp.	Anticancer
FATTY ACIDS		
Eicosapentaenoic acid (EPA)	*Tetraselmis* sp.	Anti-inflammatory, Anti-angiogenic
Docosahexaenic acid (DHA)	*Tetraselmis* sp.	Anti-inflammatory, Anti-angiogenic
Docosapentaenoic acid (DPA)	*Nannochloropsis oculata*	Anti-inflammatory
GLYCOLIPIDS		
Monogalactosyldiacylglycerol (MGDG)	*Gymnodinium mikimotoi, Stephanodiscus* sp, *Pavlova lutheri, Stephanodiscus* sp.	Anticancer Antioxidant
Digalactosyldiacylglycerol (DGDG)	*Stephanodiscus* sp	Anticancer Antioxidant
POLYSACCHARIDES		
Sulphated extracellular polysaccharide	Diatom *Phaeodactylum tricornutum*	Anti-inflammatory Inmuno modulating
Sulphated polysaccharide		
β-(1,3)-glucan	Chlorophyte *Chlorella stigmatophora Chlorella vulgaris*	Anti-inflammatory Inmuno modulating Anticancer
Sulphated polysaccharide	Prasinophyte*Tetraselmis suecica*	Anti-inflammatory
Sulphated polysaccharide	Haptophyte*Isochrysis galbana*	Anticancer
Sulphated polysaccharide Inmunomodulating Anticancer	Rhodophyte *Porphydium* sp.	Anti-inflammatory



Sulphated polysaccharide	Dinoflagellate *Gyrodinium impudicum*	Anti-inflammatory Inmuno modulating Anticancer
Extracellular polysaccharides-Spirulan	Cyanobacteria *Arthrospira platensis*	Anticancer
PROTEIN AND PEPTIDES		
Phycobiliproteins	*Spirulina platensis* *Porphyridium* sp.	AntioxidantAnti-inflammatory Anticancer
Peptides	*Chlorella pyrenoidosa*	Antioxidant, Anti-
	Cyanobacteria	inflammatory Anticancer
OTHER COMPOUNDS		
Amides	*Lyngbya majuscule*	Anticancer
Quinones	*Calothrix* sp.	Anticancer
Phenolic compounds	*Chlorella ellipsoidea,* *Nannochloropsis* sp	Antioxidant
Tocopherols	*Porphydium* sp.	Antioxidant

Table 3. Oil content of microalgae microalga

Microalgae	Oil content (% dry weight)
Botryococcus braunii	25–75
Chlorella sp.	28–32
Crypthecodinium cohnii	20
Cylindrotheca sp.	16–37
Nitzschia sp.	45–47
Phaeodactylum tricornutum	20–30
Schizochytrium sp.	50–77
Tetraselmis suecia	15–23
Dunaliella tortiolecta	

Source: Adapted from Chisti 2007

SUGGESTED READINGS

Benemann, Jr. 1992. Microalgae aquaculture feeds. Journal of Applied Phycology, 4, 233-245.

Chisti, Y. 2007. Biodiesel from microalgae. Biotechnology Advances, 25,294-306.

Talero, E., García-Mauriño, S., Ávila-Román, J., Rodríguez-Luna, A., Alcaide, A., Motilva, V. 2015. Bioactive compounds isolated from microalgae in chronic inflammation and cancer. Marine Drugs, 13, 6152-6209

Molina Grima, E., Acien Fernandez, F., Garcia Camacho, F., Chisti, Y. 1999. Photobioreactors: Light regime, mass transfer, and scale up. Journal of Biotechnology, 70, 231-47.

Muller-Feuga, A. 2000. The role of microalgae in aquaculture: situation and trends. Journal of Applied Phycology, 12(3-5), 527-534.

Inauguration of winter school 2018 by Padma Bhushan Dr. Manju Sharma

Photo with Dr. K. Gopakumar, Formerly DDG ICAR (Fy)

Field visit to India Sea Foods

Field visit to BOS Naturals

Field visit to Accelerated Freeze Drying Co. Ltd

Photo with Dr. Meledath Govindan

Lectures and Interactive Sessions

Practical Sessions


ICAR-CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
Ernakulam North P.O., Kochi-682018, Kerala
ICAR Sponsored Winter School on Recent Advances in Bioactive Compounds from Marine Organisms and Development of High Value Products for Health Management
January 23 to February 12, 2018
CMFRI
केन्द्रीय समुद्री मात्स्यिकी अनुसंधान संस्थान
Central Marine Fisheries Research Institute
കേന്ദ്ര സമുദ്ര മത്സ്യ ഗവേഷണ സ്ഥാപനം
WELCOME
Sitting (L to R) Minimol K.C., Grace Thomas, Kajal Chakraborty (Course Director), P. Vijayagopal (Head, Marine Biotechnology Division), A. Gopalakrishnan (Director), Paulson Mathew, Sathu T., Radhakrishnan E.K.
Standing (L to R) Aswathy Elizabeth Mani, Sreemol C.K., Prima Francis, Soumya Krishnan, Minju Joy, V. Rani, Seeja Thomachan Panjikkaran, Sheneya Festus, Drishya K., Anie Y., Suja Rani S., Sindhu Issac, Teena P. Varghese, Magna Thomas, Santwana Palai, Norma Xavier Chelat, Naheef K., Satya Narayan Sahoo, Jaimin Hareshbhai Bhatt, Ajoy Saha, Senthil Kuppusamy, Kedar Shashikant Damle, Shubhajit Dhara, Midhun Dominic C.D., Manukuttan K.S., Suji Chandru, Tima Antony, Soumya Salas

Marine Biotechnology Division
ICAR-Central Marine Fisheries Research Institute
Post Box No. 1603, Ernakulam North P.O., Kochi-682 018, Kerala, India

ISBN-978-93-82263-21-0
9 78-93-82263-21-0