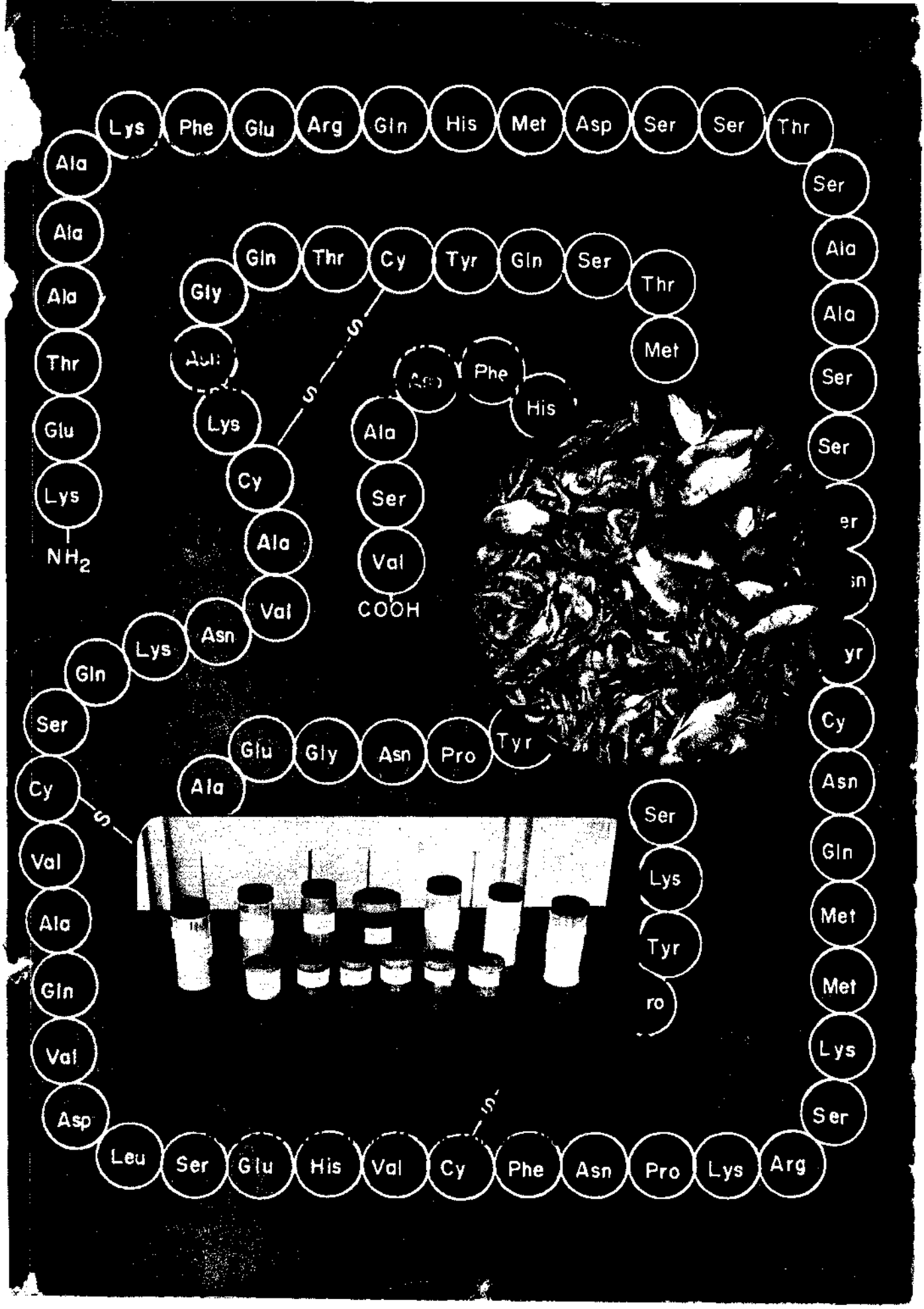
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MANUAL OF RESEARCH METHODS FOR FISH AND SHELLFISH NUTRITION

Issued on the occasion of the Workshop on
METHODOLOGY FOR FISH AND SHELLFISH NUTRITION
organised by
The Centre of Advanced Studies in Mariculture,
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Published by: **E. G. SILAS**
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COCHIN

PREFACE

The Centre of Advanced Studies in Mariculture established at the Central Marine Fisheries Research Institute has been conducting Workshops in Research Methodologies on specialised disciplines with a view to enhance the competence of the scientific workers specialising in researches connected with mariculture. The main emphasis in mariculture research has been directed towards the development of economically viable culture techniques for culturable species of fish and shellfish, with a view to augmenting the fish and shellfish production of the country. In order to develop low-cost technologies the essential operational inputs have to be rationally utilized.

It has been well established that feeding constitutes the major cost of production, often exceeding 50 per cent of the operating costs in intensive aquaculture operations. Two main factors affecting the cost of feeding are composition of the diet and efficiency of feed conversion. In order to develop least-cost formula diets of high conversion efficiency, knowledge of the nutritional requirements of the different species during the different phases of the life cycle and the nutritive value of the complex feed ingredients available in the country to the candidate species is a prerequisite.

The existing information on the nutritional requirements of cultivated species of fish and shellfish in India, is meagre and recently research has been intensified in this area. If researches on this field could be carried out using standardised experimental procedures, the data obtained on the nutritional requirements of the different species could be stored in a fish and shellfish nutrition data bank, from where data could be disseminated to the users such as feed manufacturers, farmers, extension workers and research workers as and when required. It is also necessary that the data collected on the chemical composition of the feed ingredients and their nutritive value for the species should be based on standard chemical methods and experimental procedures so that the data could be stored in

the data bank which eventually could become a National Fish Feed Information Centre.

To undertake studies on the above lines, especially by the technicians and research workers entering afresh into the field, the need of practical guides describing the research techniques and methods, planning of investigations, collection of data and their interpretation need not be emphasized. Keeping this in view, the present manual on Research Methods in Fish and Shellfish Nutrition is issued by the Centre of Advanced Studies in Mariculture on the occasion of the Workshop on Methodology of Fish and Shellfish Nutrition.

Dr. Akio Kanazawa, Professor of Nutritional Chemistry, University of Kagoshima, Japan and Consultant in Fish and Shellfish Nutrition at the CAS in Mariculture, has been kind enough to cooperate with the Scientists of CAS in Mariculture of the Central Marine Fisheries Research Institute in the preparation of this manual. There are chapters in this manual covering various methods on composition analysis of feeds, including growth inhibitors and toxins; determination of digestibility coefficient; protein evaluation; bioenergetics; determination of essential amino acid requirements using radioisotope method; research test diets for fishes and prawns; feed formulation methods; experimental design, etc. Methods of preparation of microparticulate diets, phytoplankton and zooplankton culture methods, etc. are also included to facilitate larval nutrition studies. Many of the methods given in the manual have been standardized for fish and shellfish nutrition studies in India and abroad. The users can also gain maximum benefit by suitable modifications of other methods which are given as guidelines.

I would like to thank all the scientific and technical staff especially Shri S. Ahamed Ali, Dr. K. Alagarswami, Shri D.C.V. Easterson, Shri C.P. Gopinathan, Shri T. Jacob, Shri M.S. Muthu, Dr. R. Paul Raj, Dr. A.G. Ponniah and

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CHAPTER 16

METHODS OF CULTURING PHYTOPLANKTON*

1 INTRODUCTION

It is an established fact that the success of any hatchery operation will depend mainly on the availability of the basic food, the phytoplankton. The maintenance and supply of the required species at appropriate time form a major problem facing 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, 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 in different phases of growth.

2 METHODS OF ISOLATION AND CULTURE

2.1 Methods of isolation

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

a. Pipette method:

Larger organisms can be pipetted out using micro-pipettes under a microscope.

b. Washing method or centrifugation:

Repeated washing or centrifuging the water samples results in the isolation of larger organisms.

c. By exploiting the phototactic movement:

By this method, the phytoflagellates, will move to one direction and with a micro-pipette can be isolated.

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* Prepared by C.P. Gopinathan, Central Marine Fisheries Research Institute, Cochin-18.

1. By agar-plating method:

For preparing the agar medium, 1.5% agar is added to 1 litre of suitable medium or even natural seawater and this agar solution is sterilised in an autoclave for 15 minutes under 150 lbs pressure and 120°C temperature. Now this medium is poured in sterilised petri-dishes and left for 24 hrs. In case of culture tubes, the medium is poured in 1/3 part in tubes and properly plugged with cotton before autoclaving.

The isolated species can be picked up by platinum needle or platinum loop under microscope and streaked on the surface of agar plates. After innoculation, these petri-dishes are placed in an incubation chamber for 10-15 days. Light intensity and temperature should be maintained constant. Within 15 days if the required species forms a colony, remove it by platinum loop and transfer to culture tubes half filled with suitable culture media, and subsequently from the culture tubes to culture flasks and then to 20 l glass carbuoys if the species could be cultured on a mass scale.

2.2 Culture apparatus

Vessels made 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 flasks plugged with cotton provides enough aeration. The vessels should be cleaned well and sterilized in a hot air oven.

2.3 Selection of the culture medium

On securing the desired organism, transfer the sample into a series of petri-dishes, each containing different enriched media. Keep them 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 multiply in one of the media and provide 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.

2.3.1 Culture media or solutions:

The following are some of the culture media found suitable to most planktonic algae:

2.3.1.1 Schreiber's solution:

Sodium nitrate 0.1 gm
Sodium acid phosphate 0.02 gm
Soil extract 50 cc
Filtered water 1 litre

The soil extract is prepared by boiling 1 kg good garden soil with 1 litre of distilled water in the autoclave for one hour. After 2-3 days, the supernatant liquid is separated into a flask and sterilised in an autoclave at 120°C for 20 minutes. It is advisable to keep the soil extract in a refrigerator.

2.3.1.2 Miquel's solution:

A	B
20.2 gm of Potassium nitrate dissolved in 100 ml of distilled water	Sodium phosphate -- 4 gm Calcium chloride -- 4 gm Ferric chloride -- 2.0 gm Conc. HCl -- 2 ml Dissolved in 100 ml of distilled water

To each litre of filtered seawater, 0.55 ml of A and 0.5 ml of B are added.

2.3.1.3 Conwey or Walne's medium:

This medium is mainly used for the mass culture of phytoflagellates such as Isochrysis, Tetraselmis, Monochrysis and Dicrateria species.

a. Sodium nitrate or potassium nitrate .... 100 gm
Sodium phosphate .... 20 gm
Ferric chloride .... 1.3 gm
Manganese chloride .... 0.36 gm
Bromic acid (H_3BO_3) .... 33.4 gm
EDTA .... 45 gm

Dissolve all the chemicals in one litre of distilled water. One ml is added to each litre of filtered seawater.

b. Trace metal solution:

Zinc chloride .... 2.1 gm
Calcium chloride .... 2.0 gm
Ammonium molybdate .... 2.0 gm
Copper sulphate .... 2.0 gm
Distilled water .... 100 ml

One ml is added to each litre of the seawater.

c. Vitamin stock solution:

B_{12} .... 5 mg
B_1 .... 100 mg

Dissolve in 100 ml of distilled water. 0.1 ml is added to each litre of seawater.

2.3.1.4 TMRL medium:

This solution is mainly used for the mass culture of diatoms, such as Skeletonema costatum and Chaetoceros spp.

Pottassium nitrate	....	10 gm
Sodium phosphate	....	1 gm
Ferric chloride	....	0.3 gm
Sodium silicate	....	0.2 gm

Prepare each chemical in 100 ml distilled water in separate bottles. Add 1 ml of the solution to 1 litre of filtered seawater.

2.3.1.5 P M solution:

This is another culture solution used mainly for the mass culture of diatoms, especially in 20 l glass carbuoys as a starter for the large-scale culture in bigger tanks.

Sodium nitrate	....	10 gm
Potassium phosphate	....	1 gm
Ferric chloride	....	0.2 gm
Sodium silicate	....	0.2 gm
Agrimin	....	0.1 gm
EDTA	....	0.2 gm
Thiamine (B_1)	....	0.005 gm
Cobalamine (B_{12})	....	0.005 gm

Prepare the solution in separate 100 ml reagent bottles and added to the filtered seawater 1 ml/l.

2.3.1.6 In open system (100 ton or more) the mass production of diatoms is conducted by using Commercial fertilizers:

Urea	....	100 mg/l
Agrimin	....	1 mg/l
Ferric chloride	....	2 mg/l
16-20-0	....	5 mg/l
Sodium silicate	....	2 mg/l
Potassium phosphate	....	5 mg/l

2.3.1.7 For raising a mixed culture of phytoplankters, the following chemicals can be used.

Potassium nitrate	....	1.32 gm
Sodium phosphate	....	0.66 gm
Sodium silicate	....	0.66 gm
EDTA	....	0.66 gm

Mix the chemicals in a one litre beaker, diluting with distilled water and pour to 100 l of unifiltered sea-water. Within 3 days, a bloom of diatoms could be expected.

3 GROWTH PHASES OF THE CULTURE AND HARVESTING

The usual way of the laboratory culture of the microalgae is one in which a limited volume of medium containing the necessary inorganic and organic nutrients is inoculated with a relatively small number of cells and these are exposed to suitable conditions of light, temperature and aeration. Increase in cell numbers in such a culture follows a characteristic pattern in which the following phases can be noted.

(a) Lag phase - in which no increase in cell numbers

(b) Exponential phase - in which cell multiplication is rapid

(c) Declining phase - in which the cell numbers remain constant or no more growth

(d) Stationary phase - in which the cells are stationary

(e) Death phase - in which decay may start

Harvesting of the culture is done at the exponential phase of growth. Cultures can be maintained by occasional replacement with nutrients or by regular sub-culturing.