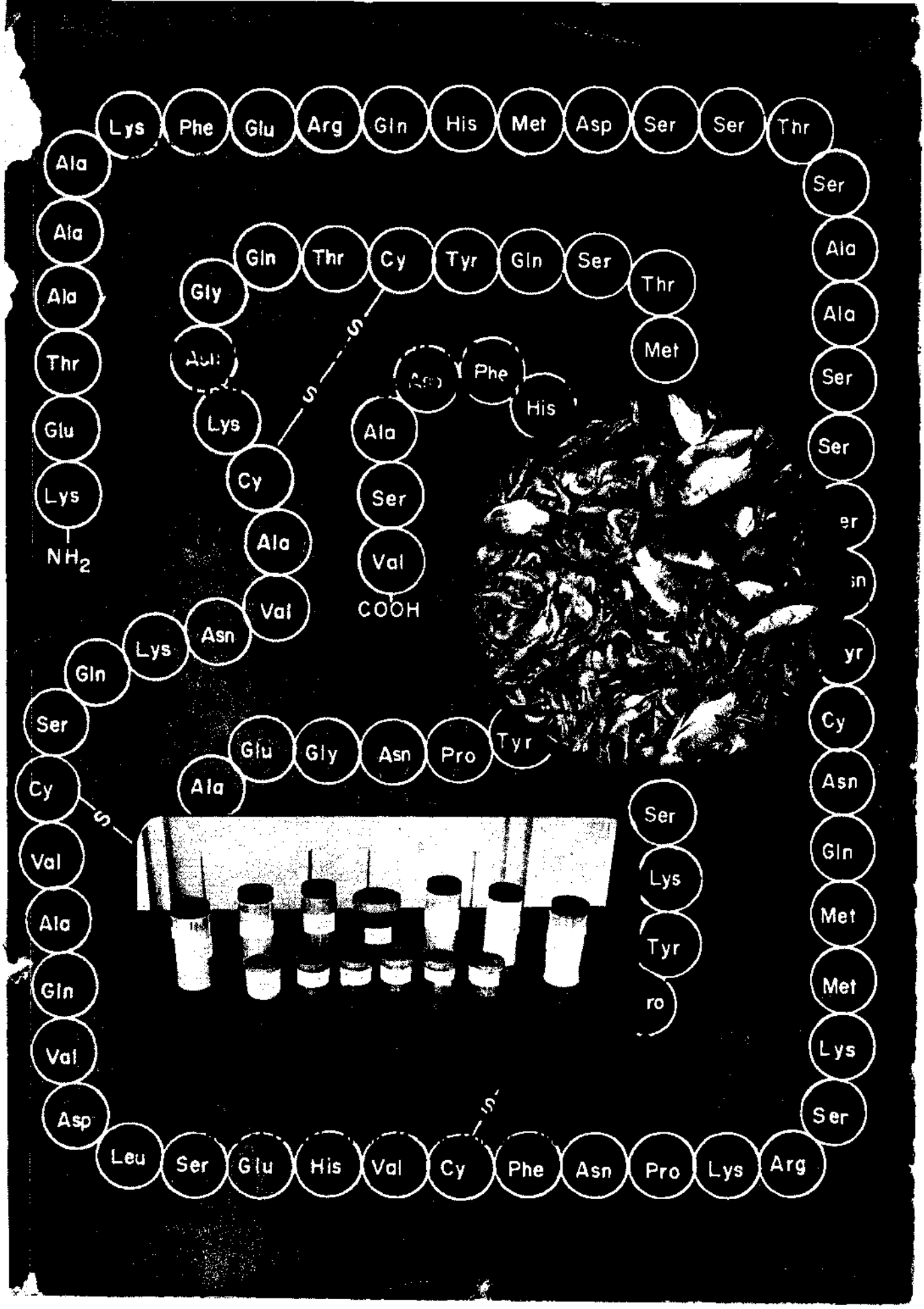
Lys Phe Glu Arg Gln His Met Asp Ser Ser Thr
Ala Ala Ala Thr Glu Lys NH2
Ser Ala Ala Ser Ser er in yr Cy Asn Gln Met Met Lys Ser
Gln Thr Cy Tyr Gln Ser Thr Met
Gly
Lys Cy Ala Val Asn Lys Gln Ser Cy Val Ala Gln Val Asp Leu Ser Glu His Val Cy Phe Asn Pro Lys Arg
Ala Ser Val COOH
Phe His
Glu Gly Asn Pro Tyr
Ala
Ser Lys Tyr ro

CMFRI SPECIAL PUBLICATION

Number 8

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,
Central Marine Fisheries Research Institute,
held at Cochin from 11 - 16 January 1982

Published by: **E. G. SILAS**
Director
Central Marine Fisheries Research Institute
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

Dr. P. Vedavyasa Rao who have rendered assistance during the preparation of this manual. Thanks are also due to Shri Johnson, Librarian and Shri Kambadkar, Technical Assistant, Central Marine Fisheries Research Institute, for the help rendered by them in printing this manual.

(E.G. Silas)
Director, CMFRI,
Sub-Project Coordinator,
Centre of Advanced Studies in Mariculture

CHAPTER 17

METHODS OF CULTURING ZOOPLANKTON*

1 INTRODUCTION

Successful hatchery production of the fry of fish and crustaceans for aquaculture purposes depends, among other things, on theavailability of zooplanktonic organisms of appropriate size for feeding the larvae. Freshly hatched Artemia nauplii have been the popular larval feed used by scientists and aquaculturists for a long time. But the high cost of Artemia cysts has lead the aquaculturists to search for other suitable zooplankters which could be easily cultured on a large scale. The rotifer Brachionus Plicatilis, the cladoceran Moina sp. the harpacticoid copepods such as Tigriopus spp and Tisbe spp, the nematode, Panagrellus sp and the cillate Fabrea salina, all of which have a high reproductive rate, short generation time, and the ability to live and grow in crowded culture conditions have been found to be useful as live feed organisms for larval rearing of cultivable species of fish and crustaceans. Among them Brachionus plicatilis and Moina sp have been most successfully used in larval rearing work in many countries and hence, in this paper, the methods of culturing these animals on a large scale are discussed in the light of experience gained in mass culturing them at the Narakkal Prawn Culture Laboratory of the Central Marine Fisheries Research Institute.

2 BRACHIONUS PLICATILIS

This filter feeding, planktonic, euryhaline, rotifer which multiplies at a very fast rate by parthenogenesis under ideal conditions has been found to be an excellent feed for rearing marine fish fry especially during the early larval stages. It is also a good food for late mysis and early post-larval stages of penaeid prawns.

---

* Prepared by M.S. Muthu, Central Marine Fisheries Research Institute, Cochin-18.

2.1 Isolation

Brachionus plicatilis grows naturally in brackishwater ponds and can be easily isolated under a binocular microscope with a fine pipette. As the rotifer is parthenogenetic, it is easy to isolate a few egg-bearing females in a petri-dish containing filtered brackishwater and by feeding them with a suitable algal food to build up a stock culture within a few days.

2.2 Culture medium

B. plicatilis is euryhaline and can be acclimatised to grow in waters with a salinity of 10 to 40 ppt. It is likely that there are geographical races which may grow better in certain salinity ranges, which will have to be determined experimentally. Generally a salinity range of 20-30 ppt is good enough. Filtered sea water can be diluted with good tap water or well water to get the desired salinity.

2.3 Culture containers

Containers ranging from 20 litre glass carbouys to 360 ton concrete tanks have been used for culturing B. plicatilis depending on the quantity of rotifers needed. For a hatchery, large plastic containers, fibreglass tanks or concrete pools will be necessary. It is essential that the containers are provided with good aeration facilities as the dense concentration of algae and rotifers consume a lot of oxygen especially during night time.

2.4 Feed for the rotifers

B. plicatilis has been grown on pure cultures of unicellular algae such as Chlorella sp., Tetraselmis sp., Dunaliella sp., bakers' yeast, marine yeast and methanol-grown yeast, and freeze-dried or spray dried Chlorella,

Spiruline or Platymonas, or even on powdered formulated diets. If fresh algae are used they are cultured separately and added to the rotifer cultures every day. To reduce the cost of culturing algae various types of agricultural fertilizers such as ammonium phosphate, ammonium sulphate and urea are used; organic manures such as extracts of chicken dung, pig manure, and groundnut oil cake have also been tried. These organic manures induced a good growth of Chlorella and bacteria which are utilized by the rotifer as food.

2.5 Culture methods

It is advisable to grow the rotifers and the algal feed in separate containers. Desired amount of algal suspension is added to the rotifer tank to maintain a concentration of 10^6 cells/ml in the case of Tetraselmis and Dunaliella or 5-10 X 10^6 cells/ml of Chlorella. Yeast, algal powders and formulated diets are given at the rate of 200-300 mg/litre per day in 3 divided doses. The rotifer population grows rapidly and attains a concentration of 200-300 nos/ml in 4-5 days after starting a culture at a temperature of 28-30°C. If the rotifer culture is not harvested, a density of upto 600 nos/ml is reached in 6-7 days and then the culture declines rapidly. It is better to harvest daily 1/3 to 1/4 of the culture and replace the volume harvested with fresh algal culture or rotifer medium. Harvests of 30-50 nos/ml per day could be made every day. Regular harvesting helps to maintain the culture in good condition for a longer period. Since the culture are not maintained under sterile conditions they are likely to be contaminated with the growth of undesirable filamentous algae and ciliates. Under such conditions the cultures should be discarded, the pools cleaned thoroughly and a fresh culture started again.

At the Narakkal Prawn Culture Laboratory of the CMFRI B. plicatilis is cultured in 24' dia. outdoor plastic pools using 20-30 ppt. brackishwater fertilized with groundnut oil cake (250 gm/ton) urea (8 gm/ton) and superphosphate

(4 gm/ton) to induce a bloom of Chlorella on which the rotifers feed.

2.6 Harvesting

Harvesting of rotifers is done by filtering through a nylon bolting silk cloth of 40 micron mesh size. The concentrated rotifers are washed in clean sea water and used for feeding larvae either in fresh or in frozen condition. Rotifers that are to be frozen should not be washed in freshwater as it leads to osmotic breakdown of cell membranes and at the time of thawing a very poor product is obtained. Harvesting should be done in the exponential growth phase when the rotifers are seen with 3 or more eggs attached to the body. The organic matter content of the body is maximum at this stage. In the senescent stage, the rotifers do not carry eggs, their body tissues are depleted, the test being practically empty and the animals swim feebly around. The nutritive value of such rotifers will be very poor indeed.

2.7 Nutritive value

It has been reported by Japanese workers that the nutritive value of the rotifers to the fish larvae depends on the diet on which the rotifers were reared. Fish larvae fed with rotifers grow on a diet of fresh algal cultures appear to be well nourished while those fed with rotifers reared on a diet of yeast or commercial single cell proteins are weak and their survival is low. This is attributed to the difference in the fatty acid composition of the rotifers fed with yeast and algae respectively. The latter are found to be rich in w3 long-chain unsaturated fatty acids (LUFA). Marine fish and crustacean larvae seem to have a specific requirement for w3 LUFA which can be satisfied only through the diet.

2.8 Maintenance of cultures

New cultures can be started from resting eggs which can be stored for long periods. B. plicatilis which normally reproduces by parthenogenesis, produces resistant

resting eggs under unfavourable conditions or when the density of the rotifer population becomes too high. B. plicatilis which have been growing in normal sea water have been induced to produce resting eggs when they are transferred to 25% seawater. By this method resting eggs could be collected and stored in a deep freeze at -14°C for 3 months and for 3 weeks in a desiccator at room temperature without loss of viability. It is reported that the shelf life of desiccated eggs can be increased to 25 weeks by a process known as sonification at low energies.

3 MOINA SP.

The freshwater cladoceran Moina is frequently found in temporary ponds. It is readily eaten by bigger fish fry and by older postlarvae of penaeid prawns. Moina also reproduces by parthenogenesis under favourable conditions and forms resting eggs through sexual reproduction under unfavourable conditions. The embryos develop inside the dorsal brood pouch and the young ones hatch out fully formed.

3.1 Isolation

Moina can be collected from ponds and a stock built up starting from a single parthenogenetic female. From a single female kept in a 2 litre beaker containing Chlorella water, it has been possible to obtain 42,000 Moina within 12 days.

3.2 Culture medium

Good tap water or well water can be used for growing Moina. Salinities above 3 ppt are not tolerated by them.

3.3 Culture containers

3' - 12' dia. plastic pools or concrete tanks can be used for Moina culture. Good aeration should be provided. The tanks are kept outdoors.

3.4 Feed for Moina

It is a filter feeder living on a variety of unicellular fresh water algae. Chlorella appears to be the best feed.

3.5 Culture methods

The culture tank is filled with freshwater, innoculated with a culture of Chlorella, fertilized with groundnut oil cake (250 gm/ton), urea (8 gm/litre) and superphosphate (4 gm/ton) and well aerated. On the second day after the water becomes slightly greenish the culture is innoculated with a pure culture of Moina which multiplies rapidly and attains a concentration of 20 to 30 thousand individuals per litre in 6-7 days. At this stage 1/3 - 1/2 the volume can be harvested and replaced by freshwater along with the proportional amounts of the above fertilizers, or by Chlorella water cultured in a separate tank using the same fertilizers. If the latter method is followed 1/3 of the culture volume can be harvested everyday. The groundnut oil cake sustains the algal bloom for a longer period and in the finely divided state may be eaten directly by the cladocerans. Chlorella blooms can be maintained by fertilizing with chicken dung or pig manure also.

Appearance of males in the culture, heralds the decline of the population by formation of resting eggs. When this happens it is better to remove all the water leaving only the sediments at the bottom and filling up the tanks with Chlorella water again. Moina culture revives in a few days.

3.6 Harvesting

Harvesting is done with a plankton net in the exponential growth phase when the females are reproducing actively by parthenogenesis. Parthenogenetic females containing 8-12 embryos in the brood pouch are rich in organic matter and are evidently more nutritive than females with resting eggs or the males. Harvested Moina are washed in water and frozen into small blocks for future use.

3.7 Nutritive value

Moina which can also be grown on yeast or commercial single cell proteins, are said to be deficient in w3 LUFA and are therefore inferior as feed for the fish and crustacean larvae. Moina fed with fresh algal cultures are nutritionally adequate.

3.8 Maintenance of cultures

Resting eggs can be collected from the bottom of the culture containers and stored in dry conditions at room temperature for 2-3 weeks without loss of viability. Fresh cultures can be started by keeping the dry resting eggs in well aerated Chlorella water; parthenogenetic females hatch out from the resting eggs within 48 hours.

4 ARTEMIA SALINA

Artemia saline nauplii are usually hatched out from cysts stored in sealed cans. Cysts hydrated in seawater hatch out in 24-30 hrs. They are isolated from the floating empty cysts by keeping a light at the transparent bottom of the conical containers used for hatching the cysts. The nauplii which congregate near the light are siphoned out and used for feeding the larvae or for starting batch cultures of adult Artemia to feed the juveniles and adults of fish or prawns. Freshly hatched Artemia nauplii are said to be nutritionally superior to one day old nauplii.

Dense batch cultures are grown in large containers using well aerated, high saline water (40-60 ppt). Suspensions of yeast, dry Chlorella or Spirulina powder, rice bran or groundnut-oil-cake-milk have been used to feed the Artemia. They become egg bearing adults in 12-14 days. The nutritional quality of the adults grown on different diets will be different and will have to be assessed in relation to the purpose for which they are raised.

Maintaining continuous cultures of Artemia under controlled conditions for cyst production is possible only on a small scale for experimental purposes. However Artemia is being cultured in salt pans for commercial production of cysts.