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# MANUAL OF RESEARCH METHODS FOR MARINE INVERTEBRATE REPRODUCTION



Issued on the occasion of the Workshop on  
**MARINE INVERTEBRATE REPRODUCTION**  
jointly organised by  
the Department of Zoology, University of Madras and  
the Centre of Advanced Studies in Mariculture,  
Central Marine Fisheries Research Institute, Cochin  
held at the University of Madras  
from 25th October to 10th November 1982

The Centre of Advanced Studies in Mariculture was started in 1979 at the Central Marine Fisheries Research Institute, Cochin. This is one of the Sub-projects of the ICAR/UNDP project on 'Post-graduate agricultural education and research'. The main objective of the CAS in Mariculture is to catalyse research and education in mariculture which forms a definite means and prospective sector to augment fish production of the country. The main functions of the Centre are to :

- provide adequate facilities to carry out research of excellence in mariculture/coastal aquaculture ;
- improve the quality of post-graduate education in mariculture ;
- make available the modern facilities, equipments and the literature ;
- enhance the competence of professional staff ;
- develop linkages between the Centre and other Institutions in the country and overseas ;
- undertake collaboration programmes ; and
- organise seminars and workshops.

Under the programmes of the Centre, post-graduate courses leading to M.Sc. (Mariculture) and Ph.D. are offered in collaboration with the University of Cochin since 1980.

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*Front cover* : SEM picture showing surface topography of *Streptocephalus dichotomus* egg.

# Manual of Research Methods for Marine Invertebrate Reproduction

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DEPARTMENT OF ZOOLOGY, UNIVERSITY OF MADRAS AND THE  
CENTRE OF ADVANCED STUDIES IN MARICULTURE, CENTRAL  
MARINE FISHERIES RESEARCH INSTITUTE HELD AT THE UNIVERSITY  
OF MADRAS FROM 25TH OCTOBER TO 10TH NOVEMBER, 1982.

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## PREFACE

The technologies of controlled reproduction, induction of spawning, sex reversal, artificial fertilisation, sterilisation and preservation of gametes are increasingly applied in aquaculture to obtain quality seed, quality fish stock and better yield. In this context, researches on different aspects of reproduction, developmental biology and physiology have assumed considerable importance besides their values in understanding of the ontogeny of the organisms. Extensive researches carried out in recent years from several laboratories in the world have not only accumulated a body of information, but also brought forth several new concepts to our understanding of the development and reproductive behaviour of finfishes and shellfishes.

In India, directed research on reproductive physiology and biology is taken up only recently and the field is still in an infant stage. In view of its emerging importance, it is identified as an area for priority research and for expertise development in the programmes of the Centre of Advanced Studies in Mariculture at the Central Marine Fisheries Research Institute, and several programmes of research are being taken up in this field with particular reference to the reproductive behaviour of the culturable finfishes and shellfishes.

Advances made on the frontiers of invertebrate reproduction in recent years have been significant enough to organise a national workshop and to prepare a manual on research methodologies for the study of the subject. Several histological, histochemical and biochemical methods and sophisticated instruments have been introduced in these studies making it essential that the scholars who desire to work and specialise in the field are given adequate basic information on the research methods so as to enable them to appreciate and advance research to understand the problems confronted in the field.

The present manual, the third in the series, is prepared and compiled by Dr. T. Subramoniam, Leader of the 'Unit of

Invertebrate Reproduction' of the Zoology Department of the University of Madras, Tamil Nadu. During the past decade, a team of research scholars are working on different aspects of marine invertebrate reproduction including the cultivable crustaceans such as *Scylla serrata*, *Panulirus homarus* and *Macrobrachium* spp. under his leadership. Contributing to our knowledge on the subject, the research results achieved so far in these aspects by the Unit have unfolded several new concepts in oogenesis, spermatogenesis, sperm transfer strategy, fertilization and endocrine control of reproduction and gamete formation.

I wish to express my great appreciation to Dr. T. Subramoniam and his team of Scholars, who by their dedication and interest evolved a series of tested research methods and set a theme of investigation through insight and skill on marine invertebrate reproduction. I am sure that this manual will be of immense use to the research scholars and scientists who would like to specialise in the subject and cognate fields.

This is the second workshop we are organising in close collaboration with the University of Madras. I wish to express my gratitude to Dr. M. Santappa, Vice-Chancellor, University of Madras for the keen interest evinced in such collaborative programmes and for the advice. I am also indebted to Dr. K. Ramalingam, Professor and Head of the Department of Zoology, University of Madras for productive discussions, continuous support and suggestions. I wish to thank Shri P. T. Meenakshisundaram and Shri K. Rengarajan, Scientists of the Central Marine Fisheries Research Institute for their help in the preparation of this manual.

E. G. SILAS,  
Director, C.M.F.R.I.

**VII. FERTILIZATION AND EARLY  
DEVELOPMENT**

## INDUCED SPAWNING IN SEA URCHINS\*

### 22.1. INTRODUCTION

The gonad wall of the sea urchin consists of an outer epithelium, the outer surface of which is bathed in the perivisceral fluid, a middle layer of conspicuous bands of smooth muscles and connective tissue and an inner layer of developing gametes with nutritive cells (Vivek Raja, 1980). The release of gametes, in nature is effected by the contraction of the muscular bands which is directly under the stimulatory effect of the radial nerve hormone (Cochran and Engelmann, 1972). The gamete discharge may be induced by acetylcholine, potassium chloride or by electrical stimulation.

The gamete discharge induced by electrical stimulation or by potassium chloride lasts as long as the stimulation continues. That induced by acetylcholine falls off abruptly a few minutes after injection but may be made long lasting by pretreatment with eserine. Gamete discharge induced by acetylcholine is inhibited by tubercurarine, hexamethonium and magnesium sulphate while electrically or potassium-induced discharge is not (Iwata and Fucase, 1964 a, b).

### 22.2 MATERIALS

Adult sea urchins (male and female).

### 22.3. REAGENTS

0.53 M Potassium chloride : Dissolve 39.5 gms of potassium chloride in 1000 ml of water.

\* Prepared and verified by P. Vivek Raja, Department of Zoology, Govt. Arts College, Nandanam, Madras-600 035.



## 22.4. PROCEDURE

### I. Direct Method

1. Select a healthy ripe sea urchin around 5 cm diameter from the rearing tank. Active movement of spines indicates the healthy condition of the specimen.
2. Measure the test diameter with the help of calipers.
3. Cut open the test with bone cutter or by scissors. Take the upper half of the animal in your hand and observe the five gonads in the inner side of aboral test.
4. With the help of forceps and small scissors remove one or two gonads.
5. Place the gonad in a Petri dish containing 20 ml of filtered sea water and tease it with the help of two fine needles. The gametes will be released into the medium.
6. Take a drop of medium along with gametes in the Pasteur pipette, place it in a slide and observe under microscope.

### II. Potassium chloride method (Tyler, 1949)

1. Select a mature healthy specimen from the rearing tank.
2. Inject 0.5 ml of 0.53 M potassium chloride into the coelomic cavity through the peristomial membrane.
3. Keep the sea urchin upside down on a 100 or 250 ml beaker which is filled with filtered sea water. Make sure that the gonopores are touching the sea water.
4. Spawning begins within a few seconds after potassium chloride injection and this process continues for a period of about 15 minutes. Take a few drops of the sea water with gametes and observe it under compound microscope.

The males are generally allowed to shed into a dry dish since the sperms keep better when undiluted. Dilute the sperm just before use.

### III. Electrical stimulation method (Iwata, 1962)

1. Select a healthy adult sea urchin from the rearing tank.
2. Keep the specimen on a beaker of sea water with its aboral side touching the rim of the beaker.

3. Place one of the lead electrodes on the test of the aboral side of the animal and the other on the moist cotton placed on the oral side of the animal and switch on the current supply (10-20 Volts AC).
4. Within a few seconds the animal starts spawning. Observe the released gametes under microscope.

Shedding begins shortly after the current is passed and actually ceases when it is interrupted. This makes the method advantageous for obtaining small amounts of eggs or sperms at repeated intervals from the same animal.

#### 22.5. OBSERVATION

Observe the structure of oocytes, ova and sperms. The mature ovum measures 70-135  $\mu\text{m}$  diameter with a small prominent nucleus of 7  $\mu\text{m}$  in diameter, without any nucleolus, whereas the oocytes possess large germinal vesicle with a prominent nucleolus. The size of the oocyte varies from 8 to 100  $\mu\text{m}$  diameter and the maximum size of germinal vesicle is 55  $\mu\text{m}$  diameter (Vivek Raja, 1980). Measure the diameter of the oocyte, germinal vesicle, nucleolus and ova with the help of micrometer. Observe the jelly coat of the ova and measure its thickness.

#### 22.6. REFERENCES

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