



CMFRI SPECIAL PUBLICATION

Number 9

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.

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

Manual of Research Methods for Marine Invertebrate Reproduction

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CENTRE OF ADVANCED STUDIES IN MARICULTURE, CENTRAL
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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 cultivable 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.

**PARTHENOGENETIC ACTIVATION AND
DEVELOPMENT IN SEA URCHIN*****23.1. INTRODUCTION**

Sea urchin eggs are ideal for parthenogenetic activation. Eversince Hertwig and Hertwig (1887) successfully induced the formation of fertilization membrane in *Paracentrotus lividus* by the treatment of chloroform, many workers have induced parthenogenetic activation in various sea urchin eggs both by physical and chemical stimuli (Harvey, 1956). Parthenogenetically activated eggs normally develop upto the pluteus stage ; but further development is reported to be very difficult (Ishikawa, 1975). In this experiment activation of sea urchin egg by double treatment with butyric acid and hypertonic sea water is described.

23.2. REAGENTS

1. *N/10 Butyric acid* : Add 9.24 ml butyric acid to 990.76 ml distilled water.
2. *2.5 M Sodium chloride* : Dissolve 146.1 gm of sodium chloride in 1000 ml distilled water.

23.3 PROCEDURE (Loeb's method, 1913)

1. Place the unfertilized eggs of sea urchin in 50 ml sea water with 2.8 ml N/10 butyric acid.
2. After 2 minutes transfer these to normal sea water.
3. After 20 minutes transfer the eggs to hypertonic sea water : i.e. a mixture of 50 ml sea water and 8 ml 2.5 M sodium chloride.

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

(Note : A mixture of 2.5 M NaCl + KCl + CaCl₂ in the proportion in which these salts exist in sea water is still better than 2.5 M NaCl, since it is less injurious).

4. 20 minutes after immersion, transfer a few activated eggs to normal sea water for every 3 minutes upto one hour.

5. Each batch of eggs should be observed under microscope. Those eggs which have been just long enough in hypertonic solution begin to cleave.

23.4 OBSERVATION

Record the changes related to the formation of fertilization membrane and cleavage. Compare the process with that of normal fertilization.

23.5. INFERENCE

When the sea urchin eggs are activated by double treatment with butyric acid and hypertonic sea water the butyric acid induces the cortical changes leading to the formation of fertilization membrane and the treatment with hypertonic sea water favours or initiates the aster formation and other changes which are necessary for the cell division (cf. Ishikawa, 1975).

23.6. REFERENCES

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