



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.

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

# Manual of Research Methods for Marine Invertebrate Reproduction

EDITED BY

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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 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.

FERTILIZATION AND EARLY  
DEVELOPMENT IN THE POLYCHAETE  
*HYDROIDES LUNULIFERA*\*

25.1. INTRODUCTION

The eggs of the marine polychaete *Hydroides lunulifera* are small and their fertilization is external. This animal is suitable for experimental studies due to a number of features such as the ease with which the sperms and eggs could be obtained, the prolonged breeding season and the relatively simple conditions under which fertilization and development are accomplished in the laboratory conditions. During cleavage the division is complete (Holoblastic); but the pattern of cleavage is spiral. During cleavage, rotational movement, occurring around the egg axis, leads to a displacement or inclination of the mitotic spindle with respect to the symmetrically disposed radii. Hence, the spiral cleavage is the result of oblique positioning of mitotic spindles in the blastomeres.

25.2. MATERIALS

Mature *Hydroides lunulifera* (males and females).

25.3. COLLECTION AND REARING THE SPECIMEN IN AQUARIA

*Hydroides lunulifera* collected from the rocky shore may conveniently be maintained in the aquaria with continuous running sea water or in the conventional fish aquaria with excess sea water and continuous aeration.

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\* Prepared and verified by P. Vivek Raja, Department of Zoology, Govt. Arts College, Nandanam, Madras-600 035, and N. Munusamy, Unit of Invertebrate Reproduction, Department of Zoology, University of Madras, Madras-600 005.

#### 25.4. PROCEDURE

1. Remove the worm from the tube by carefully cutting the base of the tube.
2. Tease the lower part of the worm to release the gametes. In mature females, the lower part of the worm is pinkish or orange in colour due to the accumulation of the ova.
3. Observe the gametes under microscope.
4. Keep the eggs and sperms in different watch glasses.
5. Wash the eggs twice in sea water and allow them to settle in a finger bowl.
6. Add one or two drops of sperm in 25 ml of sea water containing fresh eggs in a finger bowl.
7. Stir the suspension of gametes, and with a Pasteur pipette transfer 2 ml of this suspension to a small watch glass and observe under microscope.
8. Within a few minutes, the lifting off of the fertilization membrane followed by cleavage can be observed.

#### 25.5. OBSERVATION

Observe the cleavage stages : 2, 4, 8, 16, 32 and 64 cell stages ; blastula, early gastrula and late gastrula. Record the time taken for each developmental stage. Observe the cleavage planes, size and nature of arrangement of blastomeres during these stages.

#### 25.6. INFERENCE

The developing eggs are sensitive to chemicals, salinity and temperature. Study the effect of temperature, salinity and chemicals on the development of *Hydroides lunulifera* (vide Expt. No. 24).