



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
MARINE FISHERIES RESEARCH INSTITUTE HELD AT THE UNIVERSITY
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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.

**A MORPHOLOGICAL INVESTIGATION ON
THE SPERMATOPHORES OF SELECTED
CRUSTACEANS***

10.1. INTRODUCTION

In many decapod crustaceans, the male produces discrete aggregations of spermatozoa embedded in some form of protective covering, termed the spermatophores. They are transferred during mating to the oviduct or merely deposited on the sternum of the females. In this way, the spermatozoa can be retained in a viable state by the female until such time the ova are ready for fertilization. In Malacostraca, spermatophores assume a variety of shapes, and show variation in their chemical composition (Malek and Bawab, 1971 ; Uma and Subramoniam, 1979 ; Subramoniam, 1982). A correlation between the spermatophore morphology and the type of fertilization has been suggested by Spalding (1942), Uma and Subramoniam (1979) and Subramoniam (1982) for Crustacea. The present experiment aims at studying the variations occurring in the spermatophore morphology of representative crustaceans with the idea that a classification for crustacean spermatophores may be arrived at.

10.2. MATERIALS

Scylla serrata, *Clibanarius longitarsus*, *Emerita astatica*, *Albunea symmista*, *Penaeus indicus* and *Ligia exotica*.

10.3. REAGENTS

0.1% *Methylene blue* : *Vide* expt No. 8.

0.1% *Fast green* : *Vide* expt. No. 8.

* Prepared and verified by K. Uma and T. Subramoniam, Unit of Invertebrate Reproduction, Department of Zoology, University of Madras, Madras-600 005.

10.4. PROCEDURE

10.4.1. *Scylla serrata*

1. Dissect the crab and remove the mid vas deferens (MVD).
2. Collect the contents from the MVD in a watch glass.
3. Pipette out the spermatophores, settled at the bottom, and then transfer to a slide.
4. Apply a coverslip on the material, add a few drops of stain at the edges of the coverslip. Observe under microscope after 5-10 minutes.

10.4.2. *Clibanarius longitarsus*

1. Open the shell, dissect the crab and remove the reproductive system.
2. Cut a small portion of distal vas deferens. Press one end of the vas deferens using a needle and remove the spermatophoric ribbon. Keep it on a slide.
3. Add a few drops of 0.1% methylene blue. Allow it to stain for 10 minutes and apply a coverslip. Observe under microscope.

10.4.3. *Emerita asiatica*

1. Pull the fifth leg at its base with a fine forceps. This results in the removal of the entire male reproductive organs without damage (Subramoniam, 1977). Transfer it to a slide and add a drop of water. Cut the hind portion of distal vas deferens. Press one end of the cut portion using a forceps to separate the spermatophoric ribbon.
2. Stain the spermatophoric ribbon for 5 minutes. Apply a coverslip and observe under microscope.

10.4.4. *Albunea symnista*

1. Remove the carapace carefully.
2. Trace the reproductive system and separate the bulged portion of distal vas deferens. Place it in distilled water and the spermatophoric mass comes out.

3. Transfer the spermatophoric mass to a glass slide, and stain for five minutes.
4. Likewise, remove the spermatophoric mass from the distal end of the vas deferens of *Penaeus indicus* and stain.

10.4.5. *Ligia exotica*

1. Pin the animal on the board and dissect to remove the reproductive system.
2. Cut the hind part of the vas deferens and transfer it to a slide. Shake the cut end in a slide to collect the contents alone. Add a few drops of stain immediately. Apply a coverslip and observe under microscope.

10.5. OBSERVATION

Compare the morphology of spermatophores of the crustaceans you have examined. Make a classification of spermatophores based on features such as peduncle and accessory mucoid secretions. Discuss the morphological features in relation to mode of fertilization and sperm transfer.

10.6. REFERENCES

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